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

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ABSTRACT

Purpose: Caffeine enhances exercise performance but its mechanisms of action remain unclear. This study investigated its effects on neuromuscular function in a non-fatigued state and during fatiguing exercise.

Methods: Eighteen males participated in this randomised, 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, alpha-motoneuron excitability (H-reflex), corticospinal excitability, short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), silent period evoked by transcranial magnetic stimulation (SP) and plasma potassium and caffeine concentration. Immediately after baseline testing, participants ingested caffeine (6 $mg \cdot kg^{-1}$) 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 and after the fatigue protocol.

Results: Caffeine enhanced drop jump height (4.2%) and decreased SP (12.6%) in a non-fatigued state. A caffeine-related decrease in SP and SICI prior to the fatiguing activity was associated with an increased time to task failure. The participants who benefited from an improved performance on the caffeine day, reported a significantly lower sense of effort during exercise and had an accelerated post-exercise recovery of M-wave amplitude.

Conclusion: Caffeine modulates inhibitory mechanisms of the central nervous system, recovery of M-wave amplitude and perception of effort. This study lays the groundwork for future examinations of differences of caffeine-induced neuromuscular changes between those who are deemed to benefit from caffeine ingestion and those who are not.

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

NEW FINDINGS

- **What is the central question of the study?**
  What are the effects of caffeine on neuromuscular function in a non-fatigued state and during fatiguing exercise?

- **What is the main finding and its importance?**
  In a non-fatigued state caffeine decreased the duration of the silent period evoked by TMS. A caffeine-induced reduction of inhibitory mechanisms in the central nervous system prior to exercise was associated with an increased performance. Individuals who benefit from caffeine ingestion may experience lower perception of effort during exercise and an accelerated recovery of M-wave amplitude post fatigue. This study elucidates caffeine's mechanisms of action and demonstrates that inter-individual variability of its effects on neuromuscular function is a fruitful area for further work.

ABBREVIATIONS:

Central Nervous System	CNS
Intracortical facilitation	ICF
Maximal isometric voluntary contraction	MVC
Maximal M-wave	Mmax
Maximum instantaneous ascending rate of force development	RFDmax
Motor evoked potential	MEP
Motor threshold	MT
Potassium	K^+
Potentiated twitch	PTw
Rate of force development	RFD
Rate of perceived exertion	RPE
Short-interval intracortical inhibition	SICI
Soleus muscle	SOL
Stretch-shortening cycle	SSC
Superimposed twitch	SIT

TMS-evoked silent period	SP
Transcranial magnetic stimulation	TMS
Voluntary activation	VA

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; Terry E. 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 due 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, as well as 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 and Smith 2005), likely 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, since 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, 't Eijnde, & Van Leemputte, 2002; Kalmar & Cafarelli, 2006; Kalmar, Del Balso, & Cafarelli, 2006; Kalmar & Cafarelli, 1999; Kalmar & Cafarelli, 2004; 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),

so 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 potassium (K^+) levels (Lindinger, Graham, & Spriet, 1993) and attenuated muscle interstitial K^+ levels (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 (Mmax), has been demonstrated (Bowtell et al., 2018).

Effects of caffeine on the excitability of spinal motoneurones are likely but far from unequivocal: some authors have shown a positive effect in a non-fatigued state (Kalmar & Cafarelli, 1999; Walton, Kalmar, & Cafarelli, 2003; Walton, Kalmar, & Cafarelli, 2002) and during fatigue (Kalmar et al., 2006), whilst others did not observe a significant effect in a non-fatigued state (Behrens, Mau-Moeller, Heise, et al., 2015; Behrens, Mau-Moeller, Weippert, et al., 2015; 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, 2006; Kalmar & Cafarelli, 2004), both of which may contribute to enhancing 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 concurrently investigated the effects of caffeine on neuromuscular function in a non-fatigued state, during fatigue and recovery. It is also important to take into consideration that some of the aforementioned discrepant results could be partly related 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, 2009; Wiles, Coleman, Tegerdine, & Swaine, 2006). A variety of factors potentially drive this inter-individual variability 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, & 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 may not be revealed in the averaged data. Thus, as 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 further understand 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 may be responsible for any caffeine-induced ergogenic effects in a non-fatigued state and during a fatiguing stretch-shortening cycle exercise (SSC) and recovery. It was hypothesised that 1) 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 2) that caffeine would not modulate contractile properties of the muscle, but would up-regulate excitatory mechanisms and down-regulate inhibitory mechanisms in the CNS.

METHODS

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 Helsinki Declaration and its later amendments, except registration in a database.

Participants

Eighteen healthy males (age: 26.6 ± 3.1 years; body mass: 75.3 ± 7.4 kg; height: 179.3 ± 5.2 cm), who engage in recreational physical exercise, volunteered to participate in this study. The participants were non-smokers and were not overweight or obese, as 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 (6 light consumers: 21 ± 26mg; 12 regular consumers: 266 ± 125 mg), assessed by a 7-day questionnaire on intake of food and beverages containing caffeine.

Procedures

The participants visited the laboratory on 3 different occasions. The first visit was a familiarisation session where 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 increasing heights (Horita, Komi, Nicol, & Kyröläinen, 1996). The sledge was inclined at 23º for all testing with the exception of one participant, where it was necessary to incline the sledge apparatus to 27º to allow maximal rebounding. The drop-jumps were preceded by a 5-min warm-up on a cycle ergometer at an intensity of 60 to 70% of individual predicted maximal heart rate, and 10 submaximal jumps in the sledge with increasing effort. Participants visited the laboratory for two subsequent experimental days - a placebo and a caffeine condition session, in a randomised order. Participants were asked to abstain from consuming caffeine-containing foods and beverages, alcohol consumption and physical exercise within 72 h of the experimental sessions. Each experimental session began with a precapsule 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 postcapsule protocol took place 60 min after the ingestion of the capsule. The postcapsule 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 in the 3 visits.

[INSERT FIGURE 1 HERE]

Pre and postcapsule measurements

The precapsule test began with a series of stimuli applied to the tibial nerve to evoke H-reflex and M-wave recruitment curves in the soleus muscle (SOL). Transcranial magnetic stimulation (TMS) was then used to elicit evoked responses in SOL. The motor threshold (MT) was assessed, and, in a randomised order which was constant for each participant throughout the study, corticospinal excitability through unconditioned motor evoked potentials (MEP), short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were assessed. Participants then performed a warm-up identical to the one performed in the familiarisation session, followed by the last part of the neuromuscular testing which included maximal isometric voluntary contractions (MVC) 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 more than 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. Standardised 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 two seconds 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

familiarisation session. They were instructed to keep a knee angle of approximately 90º on landing, to keep their heels off of the force plate and to be as explosive as possible. These criteria were visually inspected by a research assistant. MVC, squat and drop jump trials were performed in a randomised order which was constant for each participant throughout the study, with SP being assessed immediately after MVCs.

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 where 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 familiarisation session or during the precapsule 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: rate 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 pre-determined percentage of Mmax.

RPE

RPE 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).

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° of flexion (0° = extended neutral position). The sledge was locked in an unmovable position.

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 x 10.16 cm) positioned superior to the patella (V-trodes, Mettler Electronics corp., Anaheim, USA). Square pulses with 1-ms pulse duration were applied to evoke M-waves and H-reflexes in SOL. The H and M-wave recruitment curves were generated with a series of single-pulses with interstimulus intervals that randomly ranged between 8 and 10 s, in order to avoid post-activation H-reflex depression (Crone & Nielsen, 1989). 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. Stimulation intensity was reassessed during the postcapsule test. When Mmax was reached, 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 Mmax and two squared-pulses with a duration of 1 ms and an interstimulus interval of 10 ms were applied over the isometric force plateau and approximately 2 s after each MVC.

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., Dyfed, 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 familiarisation 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.

MT was defined as the minimum intensity needed to evoke a MEP of > 200 μV in 3 out of 5 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 CV 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 randomly ranged 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 4s between trials at a test stimulus intensity of 130% of MT.

Force

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

Electromyography

Electromyographic (EMG) signals from SOL were recorded using an EISA 16-2 EMG system (EISA 16-2, University of Freiburg, Freiburg, Germany) and band-pass filtered (10-500 Hz). Oval-shaped, bipolar, pre-gelled silver chloride self-adhesive surface electrodes (AmbuBlueSensor N, Denmark, Ballerup) were placed over the mid-dorsal line of the posterior shank, below the gastrocnemius muscle. The inter-electrode

distance was 2 cm. To keep the inter-electrode resistance low (<2kΩ), 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, Cambridge, UK). A permanent marker was used to mark the location of the EMG electrodes in the familiarisation session to ensure consistent electrode placement between sessions.

Muscle soreness

Participants self-reported soreness of the triceps surae muscle on the experimental days (right 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 analogue scale from 0-10 cm, where 0 represents no soreness and 10 maximal soreness.

Blood samples

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

Serum caffeine concentrations were assessed using high performance liquid chromatography (Agilent 1100 Series HPLC equipment, Santa Clara, USA). A 5 µm, 100 x 4.6 mm reversed-phase C18 column and a 4.6 mm C18 guard cartridge insert (Phenemenex Kinetex, Phenomenex SecurityGuard ULTRA, Torrance, 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 15% to 40%) with a flow rate of 1 ml/min and a pressure of 120 to 150 bar. 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. Standards were prepared by diluting 100 mg/l 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, Torrance, USA) according to the manufacturer's instructions. Columns were conditioned by passing 1 ml of methanol and 2x1 ml of distilled water through them under vacuum. 200 µl of serum was applied and after 1 min drawn through the column under vacuum. After an equilibration period of 2 min the columns were washed with 2x1 ml of distilled water. Caffeine was eluted with 400 µl of methanol. The solvent was evaporated under nitrogen and the residue was reconstituted in 400 µl of mobile phase. K^+ was assessed by the spectrophotometric method with Konelab 20 XTi (Thermo Fisher Scientific, Vantaa, Finland).

Data analysis

The main researcher was blinded to the experimental condition 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), as well as VA and the greatest force value during MVCs were obtained through automated analysis in Spike 2 software (v6.17, Cambridge Electronic Design Limited, Cambridge, UK). Duration of SP was manually analysed in the same software. After decoding, the data obtained in each experimental session were grouped for statistical analysis.

H-reflexes and M-waves. The highest peak-to-peak amplitude of the H-reflex during the recruitment curve was determined and normalised to the peak-to-peak amplitude of Mmax. 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 normalised to the Mmax evoked at that time-point. The concomitant evoked M-wave was 7.6 ± 4.3% of Mmax on the placebo day and 7.4 ± 3.8% on the caffeine day. The Mmax value used for analysis from the pre and postcapsule measurements was the highest value evoked either during the recruitment curve protocol or before MVCs.

Motor evoked potentials (MEPs). Peak-to-peak unconditioned MEP amplitude (single pulse stimulation) were averaged from each train of 10 stimuli and normalised to Mmax. Regarding the paired-pulse paradigm (SICI and ICF) in the pre and postcapsule

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.

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

Voluntary activation (VA) and contractile properties. VA 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. VA presented from the pre and postcapsule measurements are the averaged value of the three trials. To examine contractile properties, PTw was analysed.

Force and rate of force development (RFD). MVC peak was defined as the greatest force achieved before the stimulation. In the pre and postcapsule measurements, the highest MVC peak was considered. RFD was assessed by analysing the average slope values of the force/time curve over time intervals of 0-100 and 0-200 ms, starting from the onset of contraction (identified through automated analysis in Spike 2 software), as well as the maximum instantaneous ascending rate of force development (RFDmax). For the analysis of RFD in the pre and postcapsule measurements, the trial with the highest RFDmax was considered.

Squat and drop jump performance. Jump height was determined by subtracting the participant's relative standing height 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.

Statistical analysis

Data were presented as means ± standard deviations. Statistical analysis was performed using SPSS (v20, SPSS Inc., Chicago, IL, USA). Statistical significance was set at an alpha

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 [arsin (sqrt (raw value)) – 0.2854]. The two-tailed paired Student's *t* test was used to analyse the effects of caffeine on the number of sets completed in the fatigue protocol. Pre to postcapsule data, as well as 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 current 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 were 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. Least Significant Difference (LSD) method was utilised for post hoc comparisons. For all ANOVAs, sphericity was assessed using Mauchly's test of sphericity. When sphericity was violated, Greenhouse-Geisser correction was employed.

Effect sizes were reported as Cohen's d_z for the *t* test, and as partial eta squared (η_p^2) 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.

Data availability

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

RESULTS

Plasma values of caffeine

Blood analysis revealed a non-existence of caffeine in the blood stream prior to capsule administration. Caffeine increased over time during caffeine trials [$F(1.858; 31.582) = 368.785$, $p < 0.001$, $\eta_p^2 = 0.956$], reaching an average peak level ($p < 0.001$) of 8.51 mg/l (range: 7.22 – 10.94) 60 min after capsule ingestion. Immediately after the fatigue protocol [7.04 (5.38 – 9.02)] and 6 h after ingestion [4.77 (1.78 – 7.02)] it was significantly decreased ($p < 0.001$ for both pairwise comparisons). Large inter-individual variability was observed in plasma caffeine levels throughout the protocol. Nevertheless, the degree of responsiveness to caffeine in the fatigue protocol (number of sets performed, as % change from placebo) was not significantly correlated with caffeine plasma 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 approximately 3 h after ingestion of the caffeine capsule [196 ± 29 min].

Pre- to postcapsule measurements

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 [Precapsule on the placebo day (PREPLA): 1918.6 ± 345.1 N, Postcapsule on the placebo day (POSTPLA): 1962.4 ± 374.4 N, Precapsule on the caffeine day (PRECAFF): 1893.0 ± 347.9 N, Postcapsule on the placebo day (POSTCAFF): 1898.3 ± 401.6 N]. Similarly, there was no significant effect of time, drug or time*drug for $RFD_{0\text{-}200}$ (PREPLA: 5086.3 ± 1451.9 $N \cdot s^{-1}$, POSTPLA: 5208.4 ± 1403.2 $N \cdot s^{-1}$, PRECAFF: 4676.3 ± 2077.5 $N \cdot s^{-1}$, POSTCAFF: 4875.9 ± 2059.8 $N \cdot s^{-1}$). $RFD_{0\text{-}100}$ and RFD_{MAX} behaved in the same way. Squat jump height was increased after capsule ingestion [$F(1;17) = 4.825$, $p = 0.042$, $\eta_p^2 = 0.221$], but no significant main effect of drug ($p = 0.176$) or time*drug ($p = 0.460$) were found (PREPLA: 78 ± 8 cm, POSTPLA: 79 ± 7 cm, PRECAFF: 79 ± 9 cm, 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_p^2 = 0.394] and a significant time*drug effect [F(1;17) = 59.587, p = 0.001, η_p^2 = 0.485]. Post-hoc analysis (Figure 2A) revealed a significant 4.2 ± 3.7% improvement after caffeine administration (93 ± 9 cm vs 97 ± 10 cm, p < 0.001) which was not observed in the placebo trial [93 ± 10 cm vs 94 ± 11 cm, p = 0.704].

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 H-max (PREPLA: 53.8 ± 15.3%, POSTPLA: 54.7 ± 14.4%, PRECAFF: 55.4 ± 16.6%, POSTCAFF: 58.4 ± 15.9%).

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%, 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%, POSTCAFF: 11.3 ± 5.0%), regarding time (p = 0.950), drug (p = 0.110) or time*drug (p = 0.756). Regarding SICI, there was no significant main effect of drug (p = 0.431) or time*drug (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_p^2 = 0.431, PREPLA: 32.0 ± 14.3%, POSTPLA: 38.2 ± 16.8%, PRECAFF: 31.3 ± 12.9%, 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 %, 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_p^2 = 0.243] and a significant effect of time*drug [F(1;16) = 10.764, p = 0.005, η_p^2 = 0.402]. Post-hoc analysis revealed a significant 12.6 ± 12.7% decrease of SP after caffeine ingestion (78 ± 17 vs 67 ± 12 ms, p = 0.002) which was not observed in the placebo condition (71 ± 15 vs 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.

[INSERT FIGURE 2 HERE]

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%. Pooled placebo and caffeine data revealed that PTw was significantly potentiated following three MVCs (p = 0.006, ηp^2 =0.368), but caffeine had no effect on the degree of potentiation.

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, dz = 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 to 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 Mmax 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.

The number of sets completed in the caffeine trial revealed a weak to moderate correlation with the pre to postcapsule caffeine-induced decrease in SICI (Figure 4A), as well as a significant moderate correlation with the pre- to postcapsule caffeine-induced decrease in SP (Figure 4B). The degree of responsiveness to caffeine (% 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.

[INSERT FIGURE 3 HERE]
[INSERT FIGURE 4 HERE]

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

[INSERT TABLE 1 HERE]

MVC remained depressed (Figure 5) throughout the fatiguing activity and recovery [$F(2.360; 35.394) = 22.469$, $p < 0.001$, $\eta_p^2 = 0.6$], with no significant main effect of drug ($p = 0.129$) or time*drug interaction ($p = 0.814$). Similarly, there was a significant main effect of time [$F(6;90) = 8.213$, $p < 0.001$, $\eta_p^2 = 0.354$], but not drug ($p = 0.082$) or time*drug ($p = 0.249$) for $RFD_{0\text{-}200}$. Analysis of variance for $RFD_{0\text{-}100}$ 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 [$F(2.924;40.937) = 6.683$, $p = 0.001$, $\eta_p^2 = 0.323$] but not drug ($p = 0.847$) or time*drug ($p = 0.411$). A significant main effect of time was also found for PTw [$F(6;90) = 9.089$, $p < 0.001$, $\eta_p^2 = 0.377$,], with no significant main effect of drug ($p = 0.861$) or time*drug ($p = 0.861$). VA and PTw were significantly depressed at exhaustion and throughout recovery (Figure 5).

[INSERT FIGURE 5 HERE]

Mmax was significantly depressed during fatigue and recovery. [$F(2.142;29.991) = 10.614$, $p < 0.001$, $\eta_p^2 = 0.431$], with no significant effect of drug ($p = 0.241$) or time*drug ($p = 0.591$). 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 [$F(2.938;38.196) = 2.761$, $p = 0.005$, $\eta_p^2 = 0.277$] (Figure 6).

[INSERT FIGURE 6 HERE]

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. SP was 11% lower at task failure, and remained depressed during the early stage of recovery [$F(3.011;39.138) = 3.793$, $p = 0.018$, $\eta_p^2 = 0.226$], 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 (p = 0.832) for the H-reflex measured in the ascending part of the recruitment curve.

Regarding RPE, ANOVA showed a significant main effect of time [$F(1.172;15.236) = 28.515$, $p < 0.001$, $\eta_p^2 = 0.687$] and drug [$F(1;13) = 5.698$, $p = 0.033$, $\eta_p^2 = 0.305$], but no significant interaction (p = 0.297). RPE increased throughout the protocol and pooled data from all time-points revealed significantly lower values in the caffeine condition in the first 3 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).

[INSERT FIGURE 7 HERE]

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 [$F(1;17) = 13.679$, $p = 0.002$, $\eta_p^2 = 0.446$]. In the caffeine trial, [K^+] was significantly lower [4.091 ± 0.245 vs. 3.922 ± 0.271 mmol/l, placebo and caffeine conditions for the pooled pre and postfatigue data].

For muscle soreness, there was a significant main effect of time [$F(2.729;43.665) = 21.546$, $p < 0.001$, $\eta_p^2 = 0.574$], session [$F(1;16) = 13.997$, $p = 0.002$, $\eta_p^2 = 0.467$] and a significant interaction between them [$F(2.064;33.019) = 5.078$, $p = 0.011$, $\eta_p^2 = 0.241$]. A delayed onset of muscle soreness (DOMS) 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 vs 3.3 ± 2.3, +1 day evening: 5.0 ± 2.8 vs 3.5 ± 2.5, +2 days morning: 4.9 vs 2.4 vs 2.6 ± 2.1, + 2 days evening: 3.5 ± 2.1 vs 2.0 ± 1.8).

DISCUSSION

The aim of the present study was to investigate the underlying neuromuscular mechanisms that may be responsible for any caffeine-induced ergogenic effects in a non-fatigued state and during fatiguing stretch-shortening cycle exercise (SSC). In this randomised, 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 postcapsule caffeine-induced changes in the CNS and a subsequent improvement of exercise performance. Furthermore, the subgroup of participants who responded to caffeine benefited from a decreased sense of effort during the activity and an accelerated recovery of Mmax after fatiguing exercise.

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), as well as on inhibitory and excitatory central mechanisms that were shown in the present study, may 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 (Del Coso et al., 2014; Abian et al., 2015; Pérez-López et al., 2015; Bloms et al., 2016) have actually 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, as day-to-day variability was not controlled (for example, in our study squat jump height had a day-to-day CV of 5.2%).

Effects of caffeine on the central nervous system 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 postcapsule 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. SP has usually been used as a measure of intracortical inhibition that is activated by long-lasting $GABA_B$ receptors (McDonnell, Orekhov, & Ziemann, 2006). More recently (Škarabot, Mesquita, Brownstein, & Ansdell, 2019) it has been suggested that both cortical and spinal inhibitory mechanisms may 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 may be attributed to the use of different doses of caffeine, different muscles under investigation, as well as different methodological procedures used to examine SP.

All these confounding factors may 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 which 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 may 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 actually been unable to demonstrate a relationship between neural drive and 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 this 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 may 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 may be the expression of a compensatory phenomenon to try to overcome a deficit of VA which was observed throughout the fatiguing protocol and recovery.

Carvalho et al. (2010) showed a decrease of SP (of around 17%) after a 2-min MVC in the caffeine condition 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 prior to 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, as well as after the fatigue protocol, may be due 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 prior to exercise and subsequent exercise performance was further corroborated by a weak to moderate correlation between the pre to postcapsule 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 is associated with subsequent exercise performance. At the group level, however, caffeine

did not influence the pre to postcapsule decrease in SICI, which is consistent with data obtained by Orth et al. (Orth et al., 2005) and 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 due to our inability to induce an average ratio of conditioned/unconditioned MEP amplitude above 100%. The cortical mechanisms underlying ICF are not yet fully understood, but it has been suggested that it concurrently results 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 only been reported once (Del Coso et al., 2008), which is in contrast with 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 this study, caffeine had no effect on the size of the H-reflex. This is consistent with data obtained by Kalmar et al. (2006) and by Behrens and colleagues (Behrens, Mau-Moeller, Heise et al., 2015; Behrens, Mau-Moeller, Weippert et al., 2015) but contrary to other studies where an enhancement of the peak-to-peak maximal normalised H-reflex (Kalmar & Cafarelli, 1999) and of the normalised slope of the H-reflex 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 further elucidate the effects of caffeine on spinal mechanisms. Kalmar et al. (2006) observed a protective effect of caffeine on the decline of alpha motoneuron excitability after fatigue, but this measure was unaltered in the current study. Consistent with our findings, Boerio et al. (2005) and Laurin et al. (2012) also did not observe significant changes after fatigue. H-reflexes may not be attributed to a single and isolated spinal mechanism (Zehr, 2002), and other approaches may be more sensitive to examine the effects of caffeine and fatigue on spinal excitability (e.g. cervicomedullary motor-evoked potentials - CMEPs). Unlike the evoked H-reflex, CMEPs are not affected by presynaptic inhibition (Nielsen & Petersen, 1994).

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).

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 Mmax, which remained supressed in those who did not considerably enhance their performance on the caffeine day but recovered to baseline values in those who did. These findings further support the idea of a caffeine-related enhancement of peripheral neuromuscular transmission which has previously been observed through an attenuation of plasma K^+ levels (Lindinger et al., 1993), attenuated muscle interstitial K^+ levels (Mohr et al., 2011), as well as a better preservation of Mmax 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 partly corroborated by an observed significant main effect of drug on plasma levels of [K^+], which were attenuated in the caffeine condition. Although a significant interaction effect of time*drug with a caffeine-related attenuation of K^+ accumulation in the blood stream would be expected, it could be argued that intravenous 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 blood stream resulted in an uptake of K^+ into inactive tissues (e.g. resting muscles), as noted by Lindinger et al. (1990).

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 3 trials of the fatigue protocol, whereas the other subgroup of participants did not benefit from this effect. Perception of effort may be modulated through the effects of caffeine on adenosine receptors (Doherty and 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 et al. (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 current 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. As 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.

Repeated bout effect was observed after 14 days

In this randomised, double-blind, placebo-controlled crossover trial, a lower amount of muscle soreness following the second session was observed. 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 side-story, we found that a repeated bout effect can be verified after 14 days.

Categorisation of sample participants according to the degree of responsiveness

As group mean changes may not tell the whole story, an exploratory analysis based on k-means clustering was conducted, dividing our study sample into those with a considerably high 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 due here as this classification could have led to false inferences about individuals who are deemed to respond/not respond to caffeine, due 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. 2 x the "typical error" (Ross, de Lannoy, & Stotz, 2015)]. In the current study, this approach could not be used.

Conclusions

This study elucidates the mechanisms of action of caffeine that may explain its ergogenic effects on exercise performance and contributes 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, as well as better describe the relationship between genetic polymorphisms and inter-individual variation in the response to caffeine and its neuromuscular effects.

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COMPETING INTERESTS

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Figure legends

Figure 1. Experimental protocol. Pre and postcapsule measurements were identical and included H and M-wave recruitment curves followed by transcranial magnetic stimulation (TMS). During TMS, unconditioned motor evoked potentials (MEP), 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 Mmax, 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 precapsule measurement and the postcapsule test was conducted 1 h later. The fatigue protocol took place after the postcapsule test. During the inter-series periods, neuromuscular testing was carried out, as well as at task failure and during the recovery period. Blood samples were collected immediately after the precapsule measurements, before the postcapsule test, after the fatigue protocol and exactly 6 h after ingestion (~3 h after the end of the fatigue protocol).

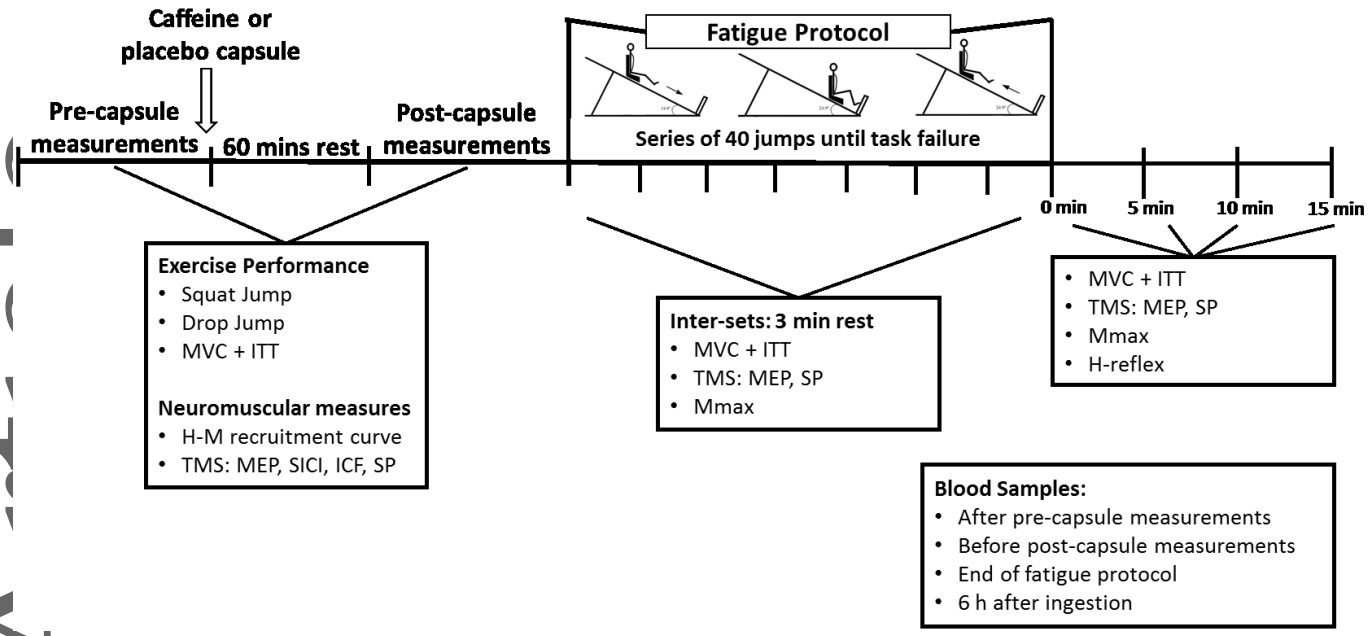

Figure 2. Pre- to postcapsule changes. **A.** Drop jump height. **B.** TMS-evoked silent period duration. Values are means (SD). Significant difference between time-points: * for p < 0.05. ♦ Significant difference between the post capsule measurement of the caffeine trial and the placebo trial, p <0.05.

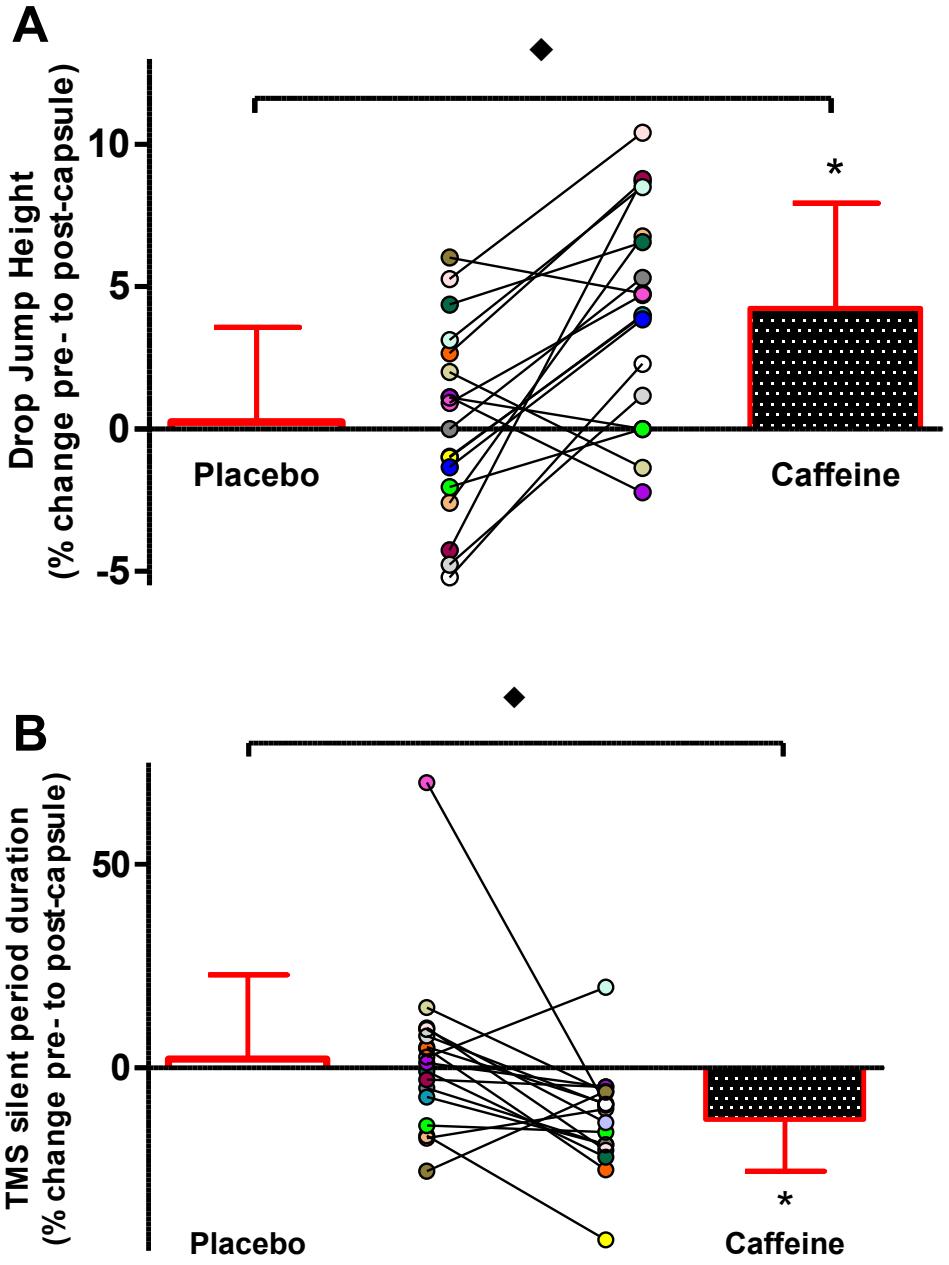

Figure 3. Percentage of change from placebo, regarding the number of sets completed for each participant. Each bar represents a different participant and positive values indicate a longer endurance time with caffeine. A k-means clustering approach was used, revealing that the lowest within cluster sum 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 to 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

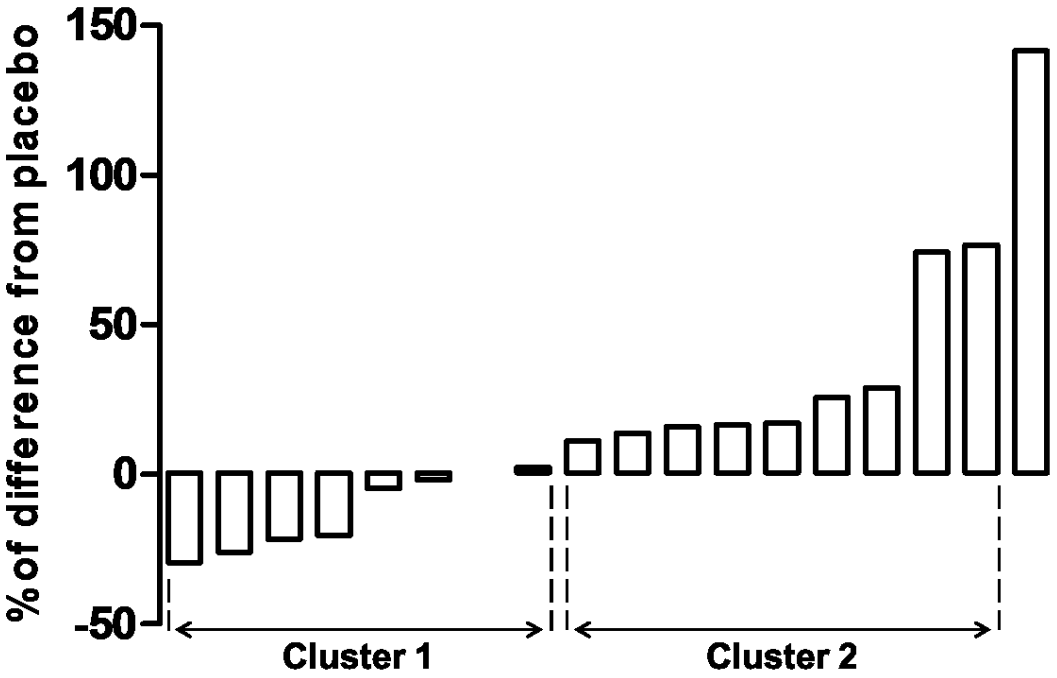

Figure 4 **A.** Correlation between pre- to postcapsule changes in short-interval intracortical inhibition (SICI) and number of sets completed during the fatigue protocol in both trials. **B.** Correlation between pre- to postcapsule changes in the duration of the corticospinal silent period (SP) and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

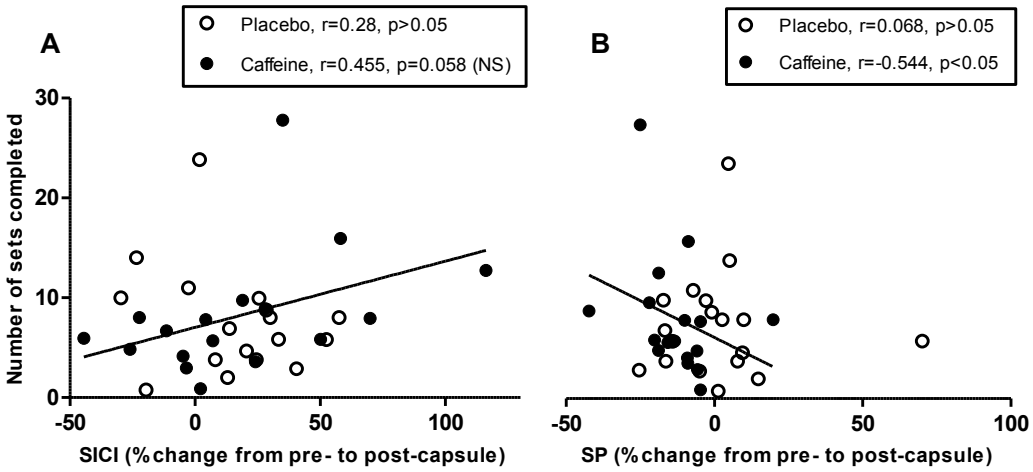

Figure 5. Changes in maximal voluntary contraction (MVC), voluntary activation (VA) and potentiated twitch (PTw) during fatigue and recovery. MVC, VA and PTw are expressed as a percentage of the postcapsule value in both trials. Values are means (SD). Significant difference from the postcapsule value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

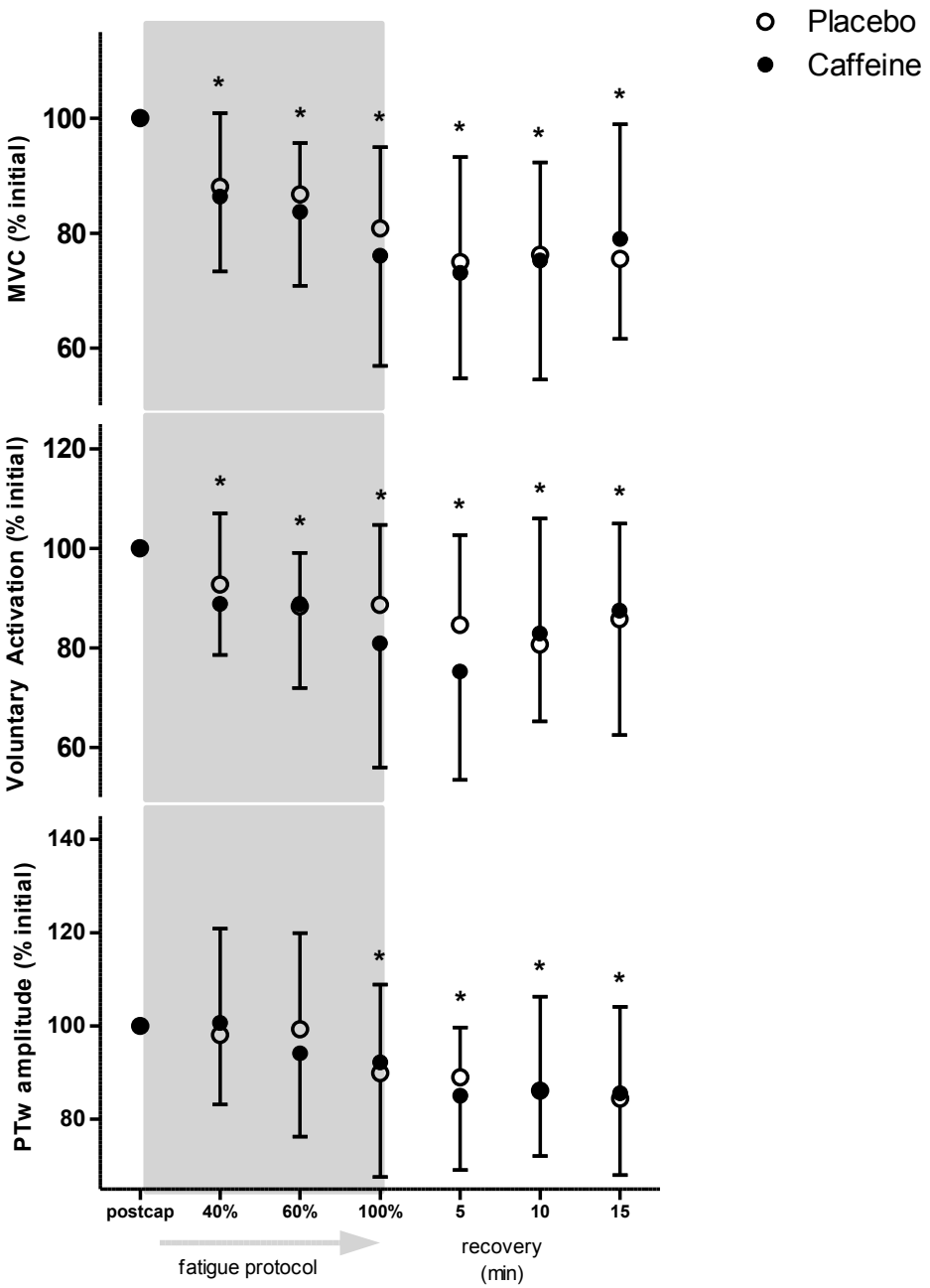

Figure 6. Changes in maximal compound muscle action potential (Mmax) during fatigue and recovery. Mmax is expressed as a percentage of the postcapsule value in both trials. Values are means (SD). Significant difference from the postcapsule value: * for $p < 0.05$. Significant difference between conditions: ♦ for $p < 0.05$.

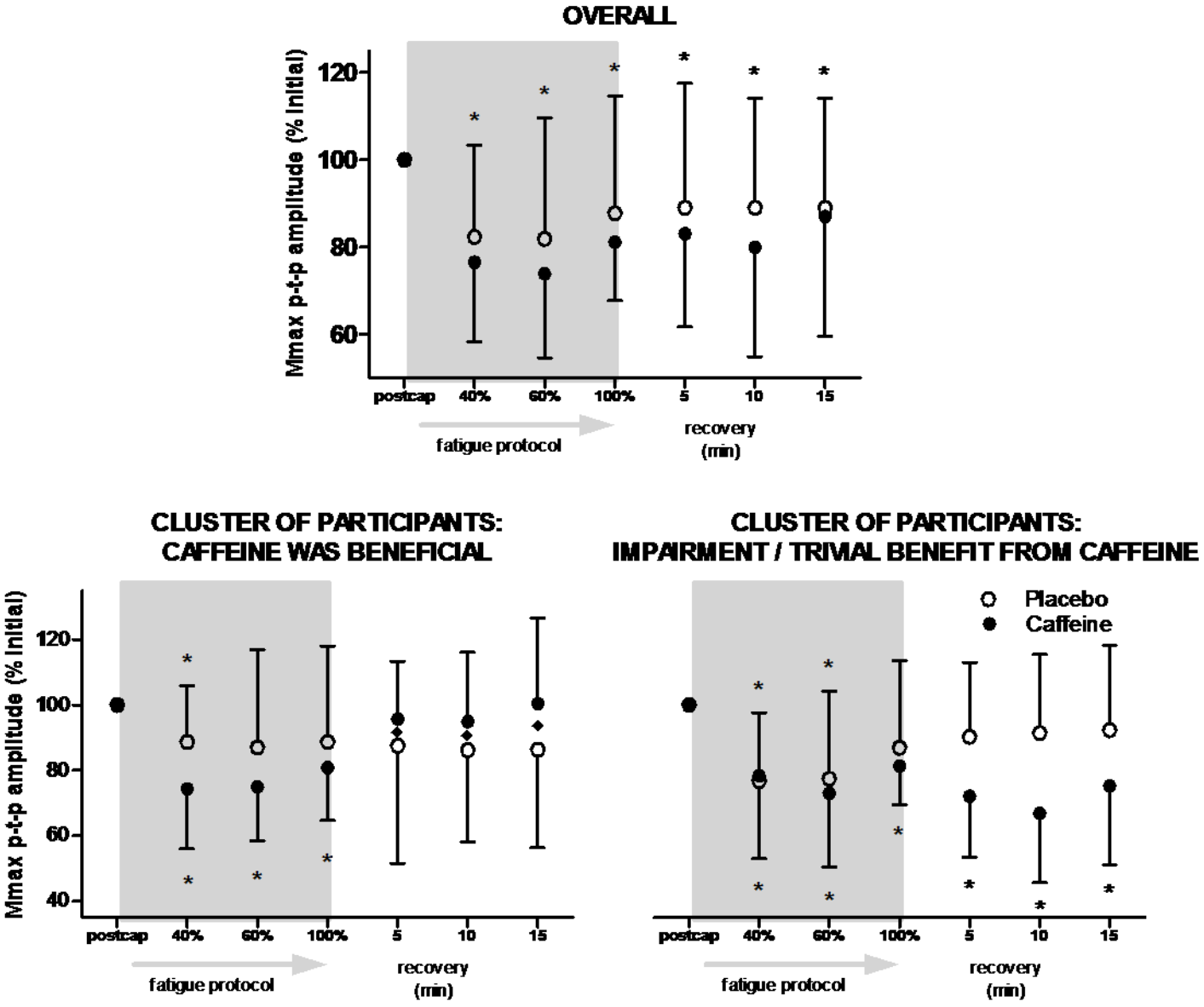

Figure 7. Changes in rate of perceived exertion (RPE) during the first sets of fatigue. ANOVA revealed a significant main effect of time and a significant main effect of drug. Post-hoc analysis showed a reduced RPE in the caffeine condition. Values are means (SD). Mixed ANOVA showed that this significant main effect of drug was present in those with observed improvements in the number of sets (above 10%) on the caffeine day (p = 0.006), but not in those with a % change below this threshold (p = 0.534).

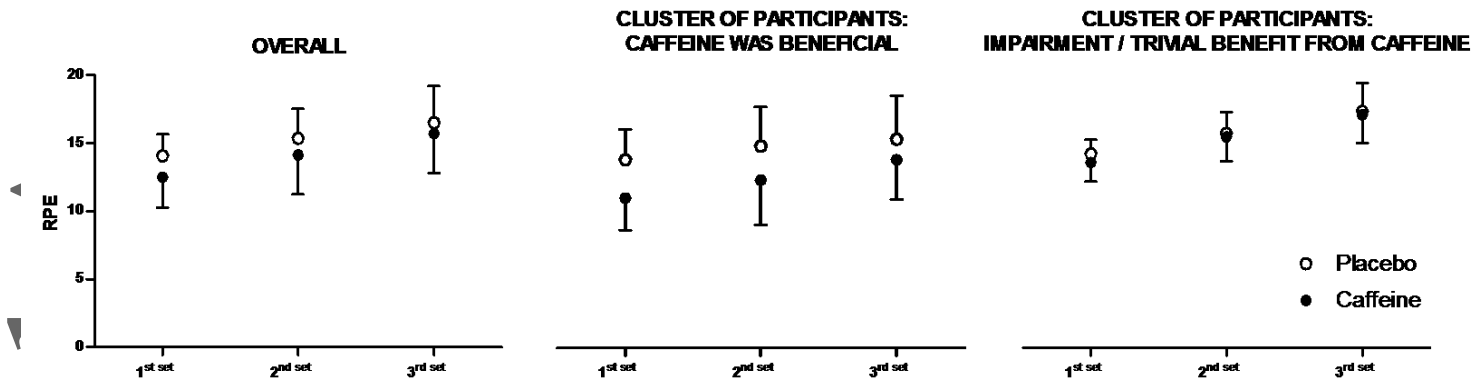

Table 1. Changes in neuromuscular variables during fatigue and recovery. Values are means ± SD. Significant difference from the postcapsule value: * for p < 0.05. When * is placed above the number, the difference was observed for the pooled placebo and caffeine data. Significant difference between conditions: ♦ for p < 0.05. MEP: motor evoked potential; Mmax: maximal compound muscle action potential; MVC: maximal voluntary contraction; RFD: rate of force development; SP: transcranial magnetic evoked silent period; VA: voluntary activation; PTw: potentiated twitch.

	Pre-fatigue	Fatigue Protocol			Recovery (min)		
		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 ($N \cdot s^{-1}$)		*	*	*	*	*	*
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 ($N \cdot s^{-1}$)			*	*	*	*	*
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 ($N \cdot s^{-1}$)		*	*	*	*	*	*
Placebo	11190.4 ± 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
Mmax (mA)							
OVERALL		*	*	*	*	*	*
Placebo	4.05 ± 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	3.38 ± 1.40	3.25 ± 1.33	3.59 ± 1.42	3.74 ± 1.73	3.60 ± 1.80	3.86 ± 1.68
Mmax (mA)							
CLUSTER (CAFFEINE: +)							
Placebo	4.18 ± 1.51	3.57 ± 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□
Mmax (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