EFFECT OF CAFFEINE ON FATIGUE DURING SUBMAXIMAL ISOMETRIC CONTRACTIONS AT DIFFERENT KNEE ANGLES

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Abstract

Introduction: In the knee extensors, the time to fatigue for intermittent isometric contractions can be increased by caffeine. Fatigue is muscle length dependent.

Aim: We examined the effect of caffeine on fatigue of knee extensors at two joint angles.

Methods: Ten male subjects (24±3 yr, 177±5 cm, 75±6 kg) with low caffeine intake (<200 mg·wk⁻¹) volunteered. Study design was double-blind and counter-balanced. Subjects were administered caffeine (6 mg·kg⁻¹) added to a non-caloric beverage or placebo, 1 hr before testing. Maximal voluntary isometric torque (MVIT) and intermittent contractions at 50%MVIT (15 s contraction, 5 s rest) were performed at knee angles of 30° and 90° (short and long length) until exhaustion. Fatigue was quantified by measurement of the MVIT 20 s post exhaustion. Surface EMG of m.vastus lateralis was analysed for root mean square (RMS). Data were analysed with 2-way ANOVA and paired t-tests with significance set at \( P<0.05 \).

Results: MVIT values were similar for caffeine and placebo at both knee angles. Time to fatigue was higher at 30° for both conditions. Caffeine increased the time to fatigue, being equal by 15% (30°) and 13% (90°). The fatigue index was similar for both conditions and knee angles.

Conclusions: Caffeine enhanced the time to fatigue during submaximal intermittent isometric contractions at different knee angles. However, potential mechanism(s) for the enhanced time to fatigue by caffeine do not seem to be muscle length dependent.

Key words: caffeine, muscle fatigue, isometric contractions, electromyography, muscle length, endurance time

Introduction

Muscle fatigue is a common experience during sport, recreational and occupational activities. Unilateral studies of skeletal muscles have shown that fatigue during sustained or intermittent isometric contractions is muscle length dependent [1, 2]. For example, the endurance time of submaximal (i.e. 50%) intermittent isometric contractions of \textit{m.quadriceps femoris} was longer at a knee angle of 30° (short muscle length) as opposed to 90° (long muscle length) [3]. The mechanisms responsible for muscle length dependent fatigue are unclear. It was suggested that differences in neural activation may exist at different muscle lengths and thus the ability to voluntary activate all motor units may vary according to length [4]. However, the central activation ratio, a measure of neural activity, was similar at both 30° and 90° [3]. Others suggested that oxygen availability may be dependent on knee angle but it was shown that even with the occlusion of blood supply, the differences in endurance were still present [3]. The number of active cross bridges and their subsequent energy consumption may be related to endurance [5]. Although De Ruiter et al. showed that muscle oxygen consumption during a sustained isometric contraction was lower at 30° compared to 90° [6], the ATP use at these different knee angles was shown to be similar [7]. Thus, it appears that mechanisms responsible for the length dependent endurance of isometric contractions are equivocal.

Caffeine is a naturally occurring plant alkaloid that crosses the blood brain barrier and is distributed in the intracellular fluid [8]. As a result of these properties, caffeine has an effect on many human tissues, including the central nervous system, the cardiovascular system, smooth and skeletal muscle [8]. The ability of caffeine to delay muscle fatigue has been studied extensively [9]. During submaximal isometric knee extension, for example, caffeine has been shown to increase time to fatigue; Kalmar and Cafarelli [10] observed a 26% increase in a sustained submaximal contraction of the quadriceps. Using intermittent protocols, both Plaskett and Cafarelli [11] and Meyers and Cafarelli [12] demonstrated a significant enhancement of the knee extensor time to fatigue with caffeine. All these studies on caffeine tested isometric endurance at a knee angle of 90°. However, because the endurance time of intermittent isometric contractions is length dependent, it is not known whether caffeine would have the same effect at different knee angles. In addition, although the caffeine's cellular mechanisms of action are currently unclear, it was originally thought that it promoted fat oxidation and thus spared glycogen [13,
14], however, the time to fatigue of short duration isometric tasks when glycogen availability is not limiting is enhanced [15]. In vitro, caffeine potentiates twitch force by increasing Ca\(^{2+}\) release from the sarcoplasmic reticulum [16]; however this occurs at dosages deadly to humans [17]. However, there is some evidence that this may occur at quantities safe for human consumption [12]. Furthermore, caffeine may be an antagonist of adenosine receptors, meaning it has the potential to increase excitatory neurotransmitter activity, and may affect voluntary activation [18].

Research suggests a knee angle dependent endurance of isometric contractions, the mechanisms of which are not currently understood, but may be related to neural activation, oxygen availability or energy metabolism [3, 4]. Caffeine has been shown to enhance endurance of submaximal isometric tasks; however whether these effects are independent of knee angle is yet to be determined. Therefore, the aim of the present study was to examine the effect of caffeine on muscle fatigue from intermittent isometric contractions at two different knee positions.

Methods

Subjects

Ten men (24 ± 3 yr, 177 ± 5 cm, 75 ± 6 kg, mean ± standard deviation, SD) who had a low self-reported caffeine intake (< 200 mg·wk\(^{-1}\)) volunteered for this study. A number of selection criteria were used to control for factors known to modify caffeine metabolism. Males were recruited, as the use of oral contraceptives can augment the half-life of caffeine [19], and throughout the luteal phase of the menstrual cycle caffeine clearance can be reduced [20]. Subjects were non-smokers, as nicotine can adjust the rate of caffeine degradation [21]. Subjects with a low caffeine intake were recruited as it was shown that chronic consumption can result in the up regulation of adenosine receptors and therefore reduce caffeine’s acute effects [22]. The study received ethical approval from the University of Chichester Ethics Committee. Subjects completed a health history questionnaire and gave written informed consent before participating.

Experimental protocol

The study had a double-blind, counter-balanced repeated measures design. Following a familiarisation session, subjects performed four experimental trials. Torque measurements were recorded using an isokinetic dynamometer (Cybex, Computer Sports Medicine Inc. Stoughton, MA). The device was equipped with full back and head support and the hips were stabilised at 90° flexion. Shoulders, hips and lower thigh were strapped to the dynamometer and the subjects were asked to fold their arms across their chest during testing. Each experimental trial was identical apart from oral administration of caffeine or placebo and the knee angle being tested; either 30° or 90° (0° = full extension). These angles were chosen so that there was a difference in muscle length [1] and comparable to previous research [3]. The right leg was used for all testing. All trials took place at the same time in the morning and were separated by a minimum of four days to assure caffeine washout [23]. Subjects refrained from strenuous activity and consumption of alcohol or caffeine in the 48 h prior to each experimental trial. This was verbally confirmed by each participant before each trial.

One hour prior to each experimental trial, pharmaceutical grade caffeine (6 mg·kg\(^{-1}\) Blackburn Distribution Ltd., Nelson, UK) added to a non-caloric beverage (Sugar Free Ribena, GlaxoSmithKline, UK) or the non-caloric beverage alone were provided to the subject. The non-caloric beverage was chosen in order to disguise the taste of the caffeine and served as the placebo. Caffeine preparation was completed by someone not involved in the study to ensure the double blind design, the same person decided the order of administration. An hour after supplementation, to allow for peak plasma caffeine levels [24], subjects partook in the experimental protocol (Fig. 1). In brief, each experimental trial consisted of 3 maximal voluntary isometric contractions, intermittent isometric contractions at 50% intensity until exhaustion and a maximal voluntary isometric contraction 20 second following exhaustion. Maximal voluntary isometric contractions lasted 3-5 seconds, were separated by 3 min recovery and averaged.

![Figure 1. Overview of the protocol. MVIT, maximal voluntary isometric torque. The protocol was performed with the m.quadriceps femoris at knee angles of 30° and 90°.](image)

The intermittent isometric contractions at 50% intensity were performed at knee angles of 30° or 90°. Contractions were held for 15 s with a recovery time of 5 s [3] until the target force fell < 45% for > 2 s. Time to fatigue was calculated from the beginning of the first contraction to the point of torque failure. Subjects were given strong verbal encouragement to continue for as long as possible. This protocol was selected because it had previously been used in studies to examine the effect of caffeine on fatigue [12] and muscle length-dependent fatigue [3]. Twenty seconds after the cessation of the fatigue protocol a final MVC was performed to allow the calculation of the fatigue index:

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\text{Fatigue index (\%) = 100\% - \frac{MVIT_{\text{post20}}}{MVIT_{\text{pre}}} \times 100\%}
\]

where MVIT\(_{\text{post20}}\) is the maximal voluntary isometric contraction (MVIC) 20 seconds after the fatigue protocol and MVIT\(_{\text{pre}}\) the maximal voluntary isometric contraction 20 second following exhaustion.
Surface electromyography

Surface electromyographic recordings of the m.vastus lateralis (VL) and m.biceps femoris (BF) were recorded with bipolar Ag/AgCl surface electrodes (Delsys, Boston, MA) during the fatigue protocol. The surface electromyography (EMG) was used to monitor muscle activation as it may be a potential mechanism of caffeine’s ergogenic effect [25]. The VL was chosen because it is an agonist of knee extension and has been used previously in caffeine studies [12]. The BF was selected as it is an antagonist to the VL so that changes in co-activation could be measured [3]. After the skin was shaved, roughened, and cleaned with 70% ethanol, electrodes were placed on the muscle belly in a bipolar fashion parallel to the muscle fibre direction with an inter-electrode distance of 10 mm. The exact placement of the electrodes followed the recommendations of the Surface Electromyography for the Non-Invasive Assessment of Muscles (http://www.seniam.org) [26]. For the VL, the electrodes were placed at two-thirds the distance on the line from the anterior spina iliaca superior to the lateral side of the patella. For the BF electrodes were placed halfway between the ischial tuberosity and the lateral epicondyle of the tibia. A reference electrode was placed on the right patella.

Data analysis

The EMG signals were amplified (100 times), digitised (1 kHz) and stored on hard disk, and then band pass filtered (10-400 kHz) and the root mean square (RMS) calculated (EMGWorks, Delsys, Boston, MA). The EMG activity (RMS value) was averaged over the first 15 s and last 15 s periods, the activity at fatigue (last 15 s) was expressed as a percentage of the first 15 s and then compared between conditions. Due to technical difficulties, EMG was only recorded for six subjects.

Statistical analyses

Data are presented as mean ± standard deviation (SD). Tests for significance were performed using two-way (supplement x knee angle) repeated measures ANOVA (SPSS v16, Chicago, USA). If significant main effects were revealed by ANOVA, Student’s paired t tests with a Bonferroni stepwise correction were applied. Caffeine results during the fatigue protocol were expressed as a percentage of the placebo values for both angles, and an additional paired t test was used to determine any differences between the two angles. Statistical significance was set at P < 0.05.

Results

Baseline values for maximal voluntary isometric torque of the m.quadriceps femoris were not altered by administration of caffeine (30º placebo: 152.2 ± 30.2 Nm; 30º caffeine: 151.4 ± 29.0 Nm, 90º placebo: 151.9 ± 28.9 Nm; 90º caffeine: 154.5 ± 28.2) (F(t, 9) = 1.606, P = 0.237). In addition, there were no differences in maximal voluntary isometric torque between knee angles (F(t, 9) = 0.821, P = 0.389) and no interaction effect (F(t, 9) = 6.106, P = 0.066).

The time to exhaustion was significantly longer at a knee angle of 30º (Figure 2) (P<0.01). At both knee angles, there was an increase in time to exhaustion following administration of caffeine (Figure 2) (P<0.01). However, the percentage increase in time to exhaustion of 15 ± 0.1% at a knee angle of 30º and 13 ± 0.1% at a knee angle of 90º were not different from each other (t(9) = 1.290, P = 0.229).

The percentage changes in RMS values for the m.vastus lateralis were not different between placebo and caffeine (30º placebo: 154.0 ± 16.2%; 30º caffeine: 153.5 ± 13.6%, 90º placebo: 157.5 ± 13.8%; 90º caffeine: 159.5 ± 11.7%) (F(t, 9) = 3.375, P = 0.629). In addition, there were no differences between knee angles (F(t, 9) = 4.342, P = 0.092) and no interaction effect (F(t, 9) = 0.770, P = 0.420). Similarly, the percentage changes in RMS values for the m.biceps femoris showed no difference between placebo and caffeine (30º placebo: 107.5 ± 4.1%; 30º caffeine: 106.8 ± 3.4%, 90º placebo: 108.1 ± 4.0%; 90º caffeine: 108.5 ± 3.6%) (F(t, 9) = 0.056, P = 0.822), no differences for knee angle (F(t, 9) = 0.611, P = 0.470) and no interaction effect (F(t, 9) = 0.254, P = 0.636).

The fatigue index for the m.quadriceps femoris from intermittent isometric contractions at 50% of maximal torque was not altered by administration of caffeine (30º placebo: 19.6 ± 1.9%; 30º caffeine: 19.8 ± 1.7%, 90º placebo: 22.1 ± 1.4; 90º caffeine: 23.1 ± 2.1) (F(t, 9) = 0.458, P = 0.516). In addition, there was no difference in fatigue index between knee angles (F(t, 9) = 27.131, P = 0.081) and there was no interaction effect (F(t, 9) = 1.052, P = 0.332).

Figure 2. Time to exhaustion for placebo (C) and caffeine (C) trials at both knee angles. *, different with placebo (P < 0.05); **, different with placebo or caffeine at 30º (P < 0.05). Values are mean ± SD.
Discussion
The aim of the present study was to determine if the effect of caffeine on isometric endurance of the m.quadriceps femoris was dependent on knee angle. Our results show 1) greater time to exhaustion at a knee joint angle of 30° compared to 90°, 2) caffeine enhanced the time to exhaustion compared to placebo at both knee angles, and 3) there was no difference in the effect of caffeine between knee angles. The novel finding of the present study is that caffeine has a similar enhancing effect on time to exhaustion at both 30° and 90° during intermittent isometric contractions at 50% of maximal voluntary isometric torque of the m.quadriceps femoris.

Effects of caffeine on time to exhaustion
Following caffeine ingestion, we observed a 15% and 13% increase in time to exhaustion at knee angles of 30° and 90°, respectively. The absolute times to exhaustion at a knee angle of 90° were similar to the work by Plaskett and Cafarelli [11] and Meyers and Cafarelli [12]. Plaskett and Cafarelli [11] demonstrated an increase of 17% at 90° using a protocol that was similar to the one used in the present study. Meyers and Cafarelli [12] showed a 21% increase in time to exhaustion following caffeine. Moreover, Kalmar and Cafarelli [10] observed a 26% increase using an exercise model of sustained submaximal contraction of the m.quadriceps femoris suggesting caffeine has a greater effect on sustained contractions. It has been shown that after an acute dose of caffeine, the magnitude of physiological responses [16] and ergogenic influences among subjects has noticeable variability [27]. This may explain why Meyers and Cafarelli [12] showed a greater enhancement with caffeine. They removed a number of subjects, termed ‘nonresponders’ from the analysis, based on the fact they showed no changes in contractile properties; when included caffeine’s effects were masked. These studies together reiterate the need for large subject numbers, and to try to control for factors that may affect acute responses to caffeine. Although no other studies have looked at time to fatigue at a 30° angle, the results of the present study appear to be in line with the research that examined fatigue at a knee angle of 90°, in that time to fatigue is enhanced.

One of the foremost mechanism thought to explain caffeine’s ergogenic action is the antagonism of adenosine receptors [18]. Adenosine, by binding to receptors in the central nervous system, inhibits neuronal firing rates and neurotransmitter release [28, 29]. Increased motoneuron excitability and self-sustained firing rates have been reported after caffeine administration [30]. In addition, caffeine ingestion was shown to increase voluntary activation and thus maximal strength [10]. However, some studies disagree with these findings, both Kalmar and Cafarelli [10] and Tarnopolsky and Cupido [31] reported no significant effects of caffeine on voluntary activation or voluntary strength. The results of the present study showed that caffeine did not enhance the torque by an unfatigued MVC compared to the placebo condition, and thus may not have affected voluntary activation. Also, the EMG findings during the fatigue protocol agree with research opposing adenosine receptor antagonism as there were no differences between conditions. This is supported by Meyers and Cafarelli [12], who not only found no significant effect of caffeine on EMG amplitude but also reported that firing rates did not decline during intermittent contractions in the control conditions and therefore the increase in endurance time in the caffeine condition could not be explained by changes in firing rates. Although neural activation was not directly measured in the present study it would appear an alternate mechanism may be responsible for the enhanced time to fatigue with caffeine.

In highly motivated subjects, exhaustion of intermittent submaximal isometric contractions is most likely due to failure of elements distal to the neuromuscular junction, specifically impairment of excitation-contraction coupling [11, 32]. Excitation-contraction coupling failure is thought to be related to disturbances in Ca²⁺ release from the sarcoplasmic reticulum [16]. In an in situ muscle preparation, caffeine increases Ca²⁺ release via the ryanodine receptors, which in turn potentiates twitch force, however, this effect occurs at dosages of caffeine that are potentially lethal to humans [16]. Nonetheless, Tarnopolsky and Cupido [31], using 20 Hz stimulation, showed an increase in force generation of the tibialis anterior with caffeine. Likewise, Lopes et al. [33], using 20-50 Hz stimulation, found an increase in tetanic force of the adductor pollicis after caffeine consumption. In both studies, there were no data on the influence of caffeine on a single twitch, increases in calcium flux induced by caffeine may have been small and intracellular Ca²⁺ concentrations may have only been changed sufficiently to alter performance after repetitive stimulation. In support of this, Tarnopolsky and Cupido [31] demonstrated that caffeine maintained evoked force approximately midway through an involuntary fatigue protocol. The results of the present study may also be support of this, as there was no effect of caffeine until the fatigue protocol. These results suggest that caffeine may alter Ca²⁺ handling and force production once a certain degree of fatigue has occurred. During progressive fatigue maximal peak twitch amplitude and the maximum instantaneous descending rate of force appear to decline, this may be a result of a reduced Ca²⁺ handling by the sarcoplasmic reticulum [12]. These results led Meyers and Cafarelli [12] to suggest that caffeine maintains Ca²⁺ reuptake and therefore improves Ca²⁺ release and force through an unknown mechanism.
Thus, these data may support the theory that caffeine has an effect on skeletal muscle that is independent of neural activation.

Effects of knee angle on time to fatigue

Endurance times were significantly longer at 30° compared to the 90° knee angle (when comparing both placebo and caffeine). This knee angle dependent endurance has previously been reported; Kooistra et al. [3] showed, when using a similar intermittent protocol, quadriceps times to fatigue of 87.8 and 54.9 s for 30° and 90°, respectively. The current time to fatigue differences between angles correspond well with other studies utilising single sustained contractions to exhaustion [1,2]. The slightly longer endurance times in the present study compared to Kooistra et al. [3] may be explained by methodological differences. Although there were similar rest periods in both studies, Kooistra et al. [3] applied full blood occlusion using a sphygmomanometer cuff. Blood flow in the current study would have increased several fold during the rest period, allowing for the removal of metabolites, theoretically this would have meant a greater removal of metabolites and their negative effects on homeostasis compared to Kooistra et al. [3] and therefore this may be reason for the longer comparative endurance times. The difference in endurance time between knee angles does not appear to originate from differences in intensity at the start of the fatigue task; the similar unfatigued MVC values between angles seem to support this.

It was previously suggested that neural activation could limit muscle endurance [4] and thus be responsible for the length dependent endurance demonstrated. However, with no differences in changes in RMS values at each knee angle, this may not be the case. The results from the surface EMG are similar to Kooistra et al. [3] who also showed no difference in VL rectified surface EMG amplitude at the point of fatigue and that co-activation was low (7.9 and 7.5 %, 30° and 90°, respectively) and did not significantly change. Kooistra et al. [3] also demonstrated that central activation ratio, used to quantify the level of neural activation, was high and similar at torque failure for both knee angles, suggesting that the central nervous system was able to maximally activate the motorneurons supplying the muscle.

Two further explanations have been given for the greater endurance at shorter muscle lengths, the first is the availability of oxygen for oxidative phosphorylation and the second is differences in energy requirement. Muscle perfusion, a factor of oxygen availability, was shown to cause differences in endurance and can alter depending on intramuscular pressure [34, 35]. This in turn can be dependent of muscle morphology or internal muscle force, which both change with knee angle [36]. However, Kooistra et al. [3], by occluding blood supply to the knee extensors, removed muscle perfusion as a potential influencing factor on endurance time. What is more, Kooistra et al. [3] showed post-exercise triplet torque was in the same range at all knee angles suggesting similar levels of contractile element fatigue. This finding agrees with the fatigue index results of the present study; in that there were no differences in fatigue index between angles. Concurrently, Kooistra et al. [3] also showed that post MVC values were similar between angles. These findings led the authors to suggest that the isometric exercise was less strenuous at shorter muscle lengths when compared to longer ones. Muscle endurance may be related to the number of active cross bridges and their corresponding energy consumption [5]. Therefore, fatigue would be at its greatest at optimum lengths where there is the greatest actin-myosin filament overlap. In frog muscle, the energy cost of contraction was shown to be lower at shorter compared to optimum lengths [37]. On the other hand, muscle energy consumption, in isolated skinned fibres and mouse muscle, was found to be similar at short and optimal lengths [38]. Yet, rich energy phosphate consumption has been shown to be lower at high and low lengths when compared with the optimum muscle length [39]. Nonetheless, both Baker et al. [7] and Sacco et al. [40] showed similar ATP use at short compared to optimum muscle lengths with nuclear magnetic resonance spectroscopy. However, more recently de Ruiter et al. [6] showed during a sustained isometric contraction lower muscle oxygen consumption at 30° compared to 60° and 90°. They concluded that this represented lower energy consumption at 30° and there would therefore be lower metabolic changes (specifically reduced pH and increased inorganic phosphate) and the lower negative consequences caused by these changes. The exact mechanisms for increased endurance times at short muscle lengths are unclear but may be related to lower energy requirements at shorter lengths.

Effects of caffeine at different knee angles

There was no difference of caffeine’s ergogenic effect between angles; both were enhanced similarly. We are not aware of other studies that examined the muscle length dependent effect of caffeine on muscle fatigue. However, in the discussion above both the increased endurance with caffeine and the greater endurance at a smaller knee angle in both conditions seem to be in line with current understandings. As the results show that caffeine had a similar effect on both knee angles it would be logical to suggest that its mechanism of action would involve something that is constant between the two lengths. Therefore, if isometric exercise at a shorter muscle length is less strenuous then caffeine may not exert its effect via the
cause of this lower energy consumption; which may be the number of active cross bridges. Subsequently, if there was lower oxygen consumption at the shorter length, it may have altered caffeine’s effects; thus, the action of caffeine may not be through pathways related to oxygen consumption. Caffeine’s effects may be mediated by changes in Ca\(^{2+}\) handling, however if Ca\(^{2+}\) handling is different at different muscle lengths, then it may not be the mediator of its enhancing effect.

Neural activation has been shown to be similar at different knee angles, as discussed previously, therefore if caffeine were to act via something that affects neural activation it would explain why there were no differences between angles. This may mean that adenosine receptor antagonism may still be the mechanism through which caffeine exerts its effect on isometric endurance. Although speculative, the present findings suggest that caffeine’s effects may be mediated by something common to both muscle lengths; however these mechanisms are unclear at this time. In the present study, the exercise model was isometric contractions, i.e. static work. However, caffeine has been shown also to be effective during isokinetic concentric contractions of the elbow flexors [41]. This work also showed that caffeine enhanced the dynamic functional properties of elbow flexors. More research is required on the mechanisms of the caffeine effect during static and dynamic contractions in addition to the efficacy of caffeine during more practical and relevant exercise models for sport and exercise performance.

We recognise that some methodological constraints could have had an impact on the results of the present study. Caffeine plasma concentrations were not measured directly, however, our dose was used in a number of similar studies involving human subjects [11, 12, 42]. Also, it has been shown that one hour is a sufficient time for plasma caffeine concentrations to peak [24]. Surface EMG activity was only recorded from one of the major knee extensors, thus preferential activation of the different heads of the quadriceps was not assessed at the different knee angles. It would appear that the greater endurance at shorter compared to longer muscle lengths is not restricted to the knee extensors; it has been reported for the m. tibialis anterior [5, 40] and elbow flexors [43]. Also, due to the task dependency of fatigue [44], further work should focus on different isometric protocols, maybe more relevant to a specific task.

In conclusion, knee angle differences exist for time to fatigue from intermittent isometric contractions, the factors that mediate these differences are open to conjecture but may include differences in neural activation or energy requirements. Caffeine enhanced the endurance time of intermittent isometric contractions of the knee extensors and these ergogenic effects appear to be independent of knee angle. Caffeine may act through one or several mechanistic mechanisms that may include antagonism of adenosine receptors or alterations in Ca\(^{2+}\) handling to increase the time to exhaustion from intermittent isometric contractions; however, the exact mechanisms are unclear.

Declaration of interest

References


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