

The effect of a physiological concentration of caffeine on the endurance of maximally and submaximally stimulated mouse soleus muscle

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Abstract The use of caffeine as an ergogenic aid to promote endurance has been widely studied, with human literature showing the greatest benefit during submaximal muscle activities. Recent evidence suggests that the acute treatment of skeletal muscle with physiological concentrations of caffeine (70 μM maximum) will directly potentiate force production. The aims of the present study are: firstly, to assess the effects of a physiological concentration (70 μM) of caffeine on endurance in maximally activated mouse soleus (relatively slow) muscle; and secondly, to examine whether endurance changes when muscle is activated submaximally during caffeine treatment. Maximally stimulated soleus muscle treated with 70 μM caffeine resulted in a significant (17.6 %) decrease in endurance. In contrast, at a submaximal stimulation frequency, caffeine treatment significantly prolonged endurance (by 19.2 %). Findings are activation-dependent such that, during high frequency stimulation, caffeine accelerates fatigue, whereas, during low frequency stimulation, caffeine delays fatigue.

Keywords Activation level · Ergogenic aid · Endurance · Power · Isolated muscle · Skeletal muscle · Work loop

Introduction

The ergogenic effect of caffeine in man has been studied extensively *in vivo*, and performance-enhancing effects have been clearly established in endurance-, strength-, and

power-based activities [1, 2]. Recent work by James et al. [3, 4] and Tallis et al. [5] were the first to demonstrate a direct effect of physiologically relevant (70 μM) caffeine concentrations on isolated mammalian muscle. However, further investigation is needed in order to assess the direct effects of 70 μM caffeine on skeletal muscle endurance.

Skeletal muscle fatigue is defined as a reduction in force-generating capacity associated with muscle activity, and is further associated with a reduced relaxation rate and changes in the force–velocity relationship [6–8]. The aetiology of muscle fatigue is influenced by the intensity and duration of physical activity and mechanistically arises from a combination of central and peripheral physiological actions [9, 10]. The primary mechanism for the given reduction in skeletal muscle force relates to a reduction in the efficiency of excitation–contraction coupling arising from a fatigue-induced impairment in sarcoplasmic reticulum (SR) Ca^{2+} release and a reduction in myofibrillar sensitivity to calcium [9, 11–15].

It has been proposed that caffeine may be effective at reducing skeletal muscle fatigue during human endurance-based activities via enhancement of excitation–contraction coupling [1, 16]. Caffeine acts as an adenosine receptor antagonistic, specifically at the A1 receptors on the skeletal muscle membrane, but can also bind directly to ryanodine receptors (RYR) of the SR [17–19]. The treatment of isolated skeletal muscle with high, millimolar, concentrations of caffeine has been demonstrated to improve Ca^{2+} handling, primarily by inducing an increased Ca^{2+} concentration in the intracellular space [20–23].

Many *in vitro* studies have demonstrated the direct force potentiating effect of caffeine using millimolar concentrations that would be toxic for human consumption [17, 24–27]. Previous findings demonstrate that the acute effect of 70 μM caffeine is greater in muscle with a relatively

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slower muscle fibre type [5]. Therefore, the present study aims to build on the previous work by James et al. [4] by being the first to assess the effects of 70 μM caffeine on the endurance of mouse soleus muscle. As the aetiology of fatigue is not only influenced by muscle fibre type but the intensity of activity and slow oxidative fibres are of primary importance during prolonged submaximal exercise, this study will uniquely examine whether the effect of caffeine is altered in soleus muscle fatigued at high compared with low frequencies of stimulation. The present study will also examine recovery from fatigue in order to determine whether caffeine treatment reduces the subsequent recovery of mouse soleus from fatigue.

Materials and methods

Dissection

The use of animals in this study was approved by the ethics committee of Coventry University. Female white mice (strain CD1 mice; Charles River, UK) were bred and kept at Coventry University. The 8- to 10-week-old mice (body mass = 30 ± 1.6 g, $n = 32$) were weighed and then killed by cervical dislocation in accordance with British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1.

Soleus muscle was isolated from the right hind limb of each mouse then pinned out at approximately its resting length at room temperature (19–21 °C). Throughout the dissection procedure, the muscle preparation was maintained in oxygenated (95 % O_2 ; 5 % CO_2) Krebs–Henseleit solution of composition (mM): NaCl, 118; KCl, 4.75; MgSO_4 , 1.18; NaHCO_3 , 24.8; KH_2PO_4 , 1.18; glucose, 10; CaCl_2 , 2.54; pH 7.55, at room temperature prior to oxygenation. For each preparation, the tendon and a small piece of bone were left attached at the proximal and distal ends. Aluminium foil T-clips were wrapped round each tendon, leaving the bone at the back of the clip, to prevent tendon slippage during force production [4].

Isometric studies

The foil clips were used to attach each muscle preparation, via crocodile clips, at one end to a force transducer (UF1; Pioden Controls, UK) and at the opposing end to a motor (V201; Ling Dynamic Systems, UK). The position of the motor arm was detected via a Linear Variable Displacement Transformer (DFG5.0; Solartron Metrology, UK).

The muscle was maintained in circulated oxygenated Krebs–Henseleit solution at a constant temperature of 36 ± 0.4 °C. In order to maintain this stable temperature for the duration of the experiment (2.5 h), a Krebs–Henseleit

solution was circulated through an external heater/cooler system (Grant LTD6G; Grant Instruments, UK) and the temperature inside the bath was continuously measured with a digital thermometer (Checktemp C; Harvard Apparatus, UK). Square wave electrical pulses of 160 mA were delivered to the preparation via parallel platinum electrodes, while the muscle was held at a constant length, to generate a series of isometric twitches. The electrodes were not in contact with the nerve branch or the fibre itself but stimulated the muscle via the fluid surrounding it.

Muscle length and stimulus amplitude (12–16 V) were optimised in order to achieve maximal isometric twitch force. The muscle length that corresponded to maximal isometric twitch force was measured using an eyepiece graticule, fitted to a microscope, and was defined as L_0 . Mean muscle fibre length was calculated as 85 % of L_0 [28]. Maximal isometric tetanic force was measured by subjecting the preparation to a burst of electrical stimuli (320 ms) at a pulse width of 1.5 ms.

Stimulation frequency was optimised to yield maximal tetanic force (usually 140 Hz), by measuring the response over a stimulation frequency range (100–160 Hz). Following this, the tetanic force response was measured at two submaximal stimulation frequencies (40 and 70 Hz). The previously optimised stimulation amplitude was kept the same throughout all tetani. A 5-min rest period was imposed between each tetanus in order to ensure that the muscle had sufficient recovery time.

Isometric tests were carried out in the same way on all muscle preparations used in this investigation. Thereafter, each muscle preparation was subjected to work loop experiments.

Work loop studies

The work loop technique assesses the ability of the muscle to produce power whilst undergoing cyclical length changes [4, 29, 30]. Here, the muscle was held at L_0 and the stimulation amplitude and stimulation frequency parameters that yielded maximal tetanic force were employed. Each muscle was subjected to four sinusoidal length change cycles per set at a total symmetrical strain of 0.10, thus the muscle lengthened by 5 % from L_0 followed by a shortening to 5 % shorter than L_0 before returning back to L_0 at a cycle frequency of 5 Hz, which represents the cycle frequency that elicits maximal power output in soleus and is attainable in vivo [28, 31]. The strain used comes from previous determination of strains that produce maximal power output at 5 Hz in soleus and that are attainable during in vivo locomotion [28, 32]. Muscle stimulation and length changes were controlled using custom written software (Testpoint; CEC, MA, USA) via a D/A board

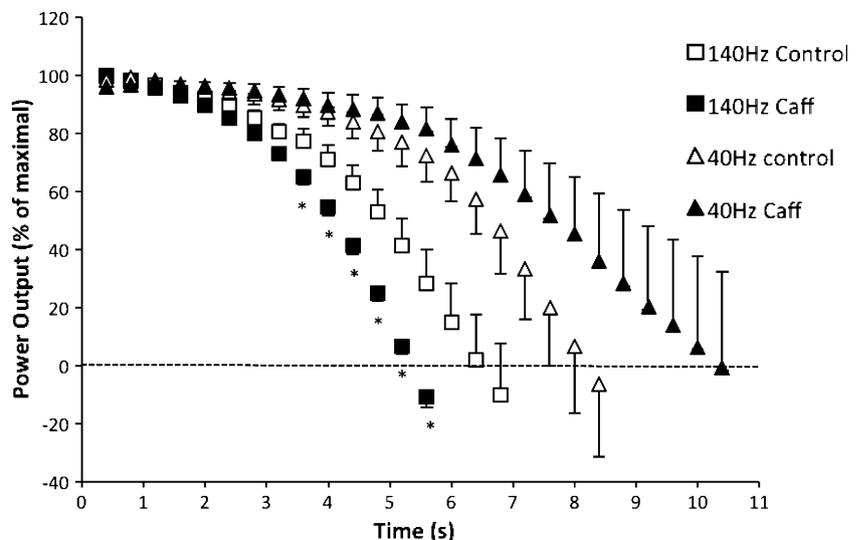
(KPCI3108; Keithley Instruments, OH, USA). Data were sampled at a rate of 10 kHz and then a work loop was formed by plotting force against length, the area of which represented the net work done by the muscle during a single length change cycle [29]. The preparations were electrically stimulated by altering burst duration until maximal net power output was achieved.

The burst duration denotes the short phase of unique stimulations that occur during the shortening phase of the work loop cycle. Each preparation was subjected to a 65-ms burst duration (8 stimuli), consistent with previous studies [3, 4, 32]. In general, this burst duration was found to elicit optimal work at both maximal and submaximal stimulation frequency. However, on occasion at 40 Hz (submaximal) stimulation frequency, the burst duration was increased to 76 ms (i.e. 1 extra stimulus). This adjustment was determined by examining the force produced and the relaxation phase of the work loop shape. James et al. [28] determined that a stimulation phase shift of -10 ms elicited maximum power output at this cycle frequency, therefore phase shift was fixed in all experiments. This stimulation phase shift dictates that stimulation of the muscle starts 10 ms prior to the muscle reaching maximal length.

Prior to the fatigue protocol, muscle power output was measured at stimulation frequencies that yielded maximal (140 Hz; high) and submaximal (40 Hz; low) power in all the preparations used at the previously defined parameters. Each muscle was subjected to sets of 4 work loops at 10-min intervals; the second loop of each set of 4 was used as an indicative measure of performance. A 10-min rest interval was enforced between each set of 4 work loops, both here and throughout the remainder of the protocol, in order to allow ample time for recovery [3]. The fatigue protocol was then conducted at one of either maximal (140 Hz) or

submaximal (40 Hz) stimulation frequency, and was composed of 4 distinct phases, the control phase, the treatment phase, the fatigue run, and the recovery phase, lasting a total of 130 min (4 work loops at 10-min intervals). During the control phase (30 min), the muscle was incubated in standard Krebs–Henseleit solution and 3 assessments of muscle power output were made in order to determine the stability of the preparation. Immediately after the control phase, during the treatment phase (40 min), the circulating fluid was changed to Krebs–Henseleit solution containing $70 \mu\text{M}$ caffeine and a further 3 assessments of muscle power output made. Ten minutes later, whilst the muscle was still incubated in $70 \mu\text{M}$ caffeine, the muscle was subjected to a fatigue run. Here, 200 consecutive work loops were delivered at a cycle frequency of 5 Hz using the same stimulation parameters used in the rest of the protocol. Fatigue was defined as the time until each muscle preparation failed to produce active work. Subsequently, muscle power output was recorded for every second loop until the muscle produced net negative work (i.e. work produced during shortening was less than the work required to re-lengthen the muscle), consequently statistical analysis for endurance uses these data (Fig. 1). Forty minutes of caffeine treatment were allowed prior to the fatigue run in order to allow sufficient time for each preparation to reach its peak response to the caffeine treatment [4, 5]. Typical work loop shapes at fixed time points during the fatigue run were plotted in order to make comparisons in the mechanical characteristics of the preparations between control and treatment muscle (Fig. 2). The experiment concluded with 6 measurements (60 min) of power output in standard Krebs in order to monitor recovery of the muscle preparation post-fatigue (Fig. 3). In order to provide an experimental control, the same protocol was followed; however, the muscle was incubated in standard Krebs–Henseleit throughout this recovery period. During the

Fig. 1 The effects of $70 \mu\text{M}$ caffeine treatment and stimulation frequency on soleus muscle power output following continues work loop stimulation. Values are displayed as a percentage of maximal work loop power output [data represented as mean and SE; $n = 8$ in each case; significant differences ($*p < 0.05$) in power output at each individual time point between 140 Hz control and 140 Hz caffeine treated]



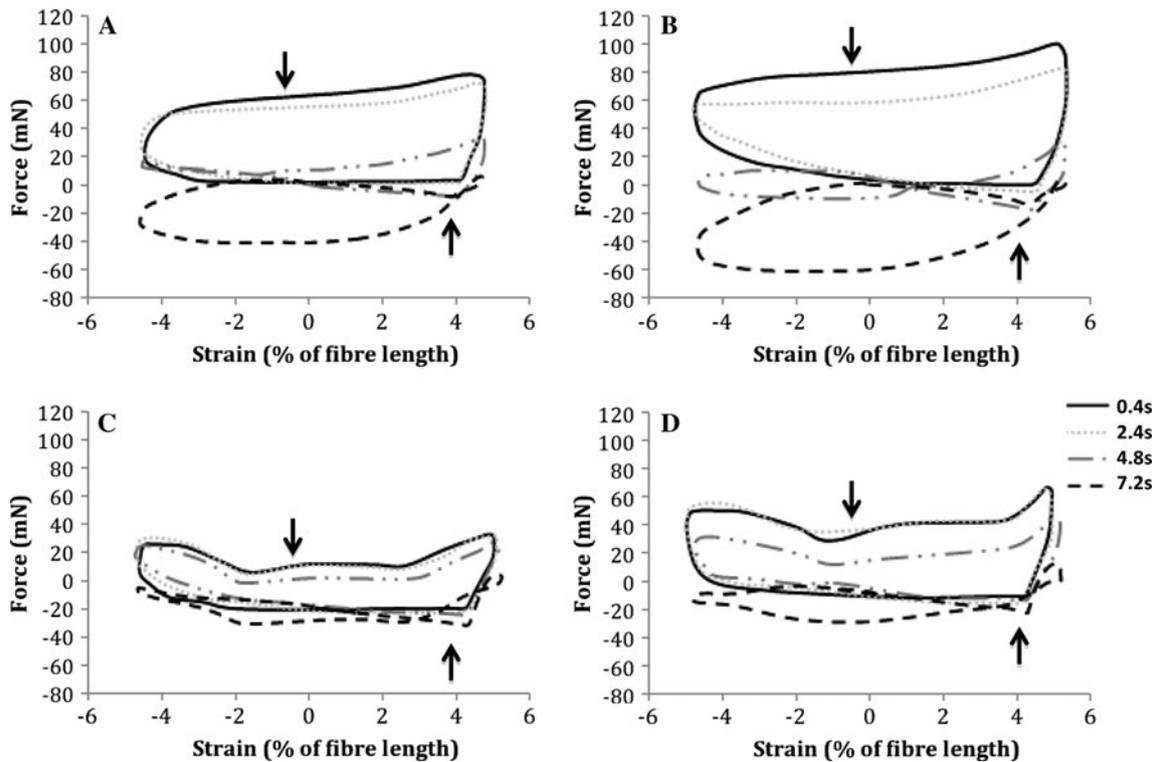


Fig. 2 Typical effects of fatigue on work loop shape for control soleus stimulated at 140 and 40 Hz (a and c, respectively) compared with those treated with 70 μ M caffeine (b 140 Hz caffeine, d 40 Hz caffeine) (arrows indicate where stimulation

typically started, towards the end of lengthening, and finished, during shortening; 0.4s, 2.4s, 4.8s and 7.2s represent time since the start of the fatigue protocol)

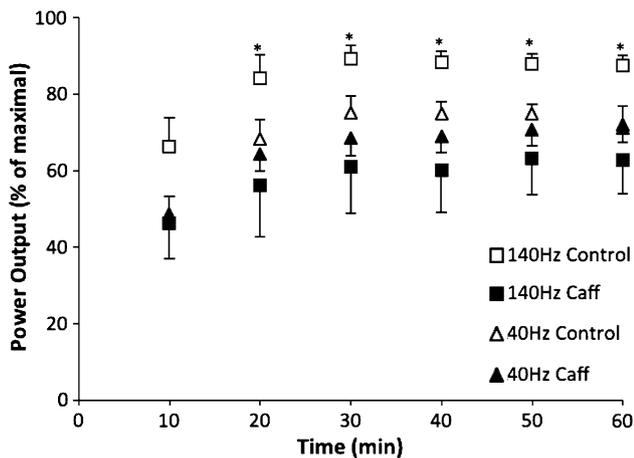


Fig. 3 Effect of 70 μ M caffeine treatment and stimulation frequency on recovery of soleus muscle following fatigue (data represented as mean and SE; $n = 8$ in each case; asterisks represent significant differences in power output at each individual time point between 140 Hz control and 140 Hz caffeine treated)

fatigue protocol, each muscle preparation was used in only one experimental condition (control or treatment) and was assessed at only one stimulation frequency (140 Hz or 40 Hz) (32 muscles in total; $n = 8$ in each case).

Muscle mass measurements and dimension calculations

At the end of the experiment, the muscle was disconnected from the apparatus and the tendons and bones removed leaving the muscle intact. Following this, the muscle was blotted on tissue paper to remove excess fluid. The muscle was then placed on an electronic balance (Mettler Toledo B204-S; Zurich, Switzerland) to determine the wet muscle mass. Mean muscle cross-sectional area was calculated from mean fibre length, muscle mass and an assumed muscle density of 1,060 kg m^{-3} [33]. Isometric stress was calculated as force divided by mean muscle cross-sectional area. Muscle power output was normalised to muscle mass to express power as watts per kilogram.

Statistical analysis of the data

Single factor analysis of variance (ANOVA) was performed in SPSS (v.16; SPSS, IL, USA) in order to investigate the effect of stimulation frequency on isometric stress and work loop power in all muscle preparations used in the study. Single factor ANOVA with Tukey post hoc tests were used to test for differences in pre treatment isometric stress and work loop power output between each experimental group (pre-treatment muscle stress and power

output for 140 and 40 Hz were compared between muscles to be used in the control protocol and muscles to be used in the treatment protocol).

A two-factor ANOVA was used to determine if the fatigue run induced a significant reduction in muscle power output over time, and to assess whether this effect was significantly different between treatment groups. Tukey post hoc tests were conducted in order to assess whether endurance was significantly different between: 140 and 40 Hz controls; 140 Hz control and 140 Hz caffeine; and 40 Hz control and 40 Hz caffeine. As caffeine treatment was shown to have a significant effect on endurance, two-sample *t* tests were conducted (Microsoft Excel 2007 version) at each time point for the same stimulation frequency in order to highlight any specific statistical difference in power output between control and caffeine-treated muscles at the same stimulation frequency (i.e. 2 *t* tests at each time point: 40 Hz control compared with 40 Hz caffeine treated, and 140 Hz control compared with 140 Hz caffeine treated).

A two-factor ANOVA with Tukey post hoc tests was conducted on the data post the fatigue run in order to test for significant differences in the muscle's ability to recover from fatigue over the 60-min duration and to test whether recovery differed between treatment categories. Therefore, treatment and time were used as the fixed factors and percentage of maximal power output the dependant variable. As before, two-sample *t* tests were conducted at each time point in order to assess specific differences in power output between control and caffeine treatments at the same stimulation frequency.

Results were interpreted as significant when $p < 0.05$. Values are displayed as mean \pm standard error.

Results

Reducing stimulation frequency from 140 to 40 Hz resulted in a significant reduction in both isometric stress (by 26 %) and work loop power output (by 51 %) in mouse soleus muscle (Table 1; ANOVA $p < 0.001$ in both cases).

Mean pre-treatment isometric stress and work loop power output at 140 Hz were not significantly different between muscles to be used in the control protocol and muscles to be used in the caffeine treatment protocol (202 ± 15 kN m⁻² and 32.4 ± 2.1 W kg⁻¹ in control, compared to 202 ± 18 kN m⁻² and 33.8 ± 2.3 W kg⁻¹ in caffeine-treated, for stress and power, respectively; Tukey $p > 0.9$ in both cases). Similarly, there was no significant difference in submaximal stress or power output between muscles to be used in the control protocol and muscles to be used in the caffeine treatment protocol when tested at 40 Hz (154 ± 20 kN m⁻² and 15.9 ± 1.9 W kg⁻¹ in control, compared to 146 ± 13 kN m⁻² and 16.6 ± 1.4 W kg⁻¹ in caffeine-treated, for stress and power, respectively; Tukey $p > 0.95$ in both cases). This confirms that the muscles were of similar quality prior to treatment, of similar quality to previous studies [3, 5], and that any subsequent effects were solely the effect of caffeine.

Effects of stimulation frequency and 70 μ M caffeine treatment on endurance

Independent of treatment, the fatigue protocol elicited a significant decrease in active power output of soleus muscle over time (Fig. 1; ANOVA $p < 0.001$). Power output over time was significantly affected by treatment group (Fig. 1; ANOVA $p < 0.001$). In control muscle, 140 Hz stimulation frequency resulted in significantly decreased endurance compared to 40 Hz (Fig. 1; Tukey $p = 0.003$). Control muscle stimulated at 40 Hz stimulation frequency maintained power output above 80 % of maximum for nearly twice as long as controls at 140 Hz stimulation frequency. After examination of individual time points, 140 Hz control muscle produced significantly greater power than 140 Hz caffeine-treated from 3.6 s of the fatigue run onwards (Fig. 1; two-sample *t* test, $df = 14$, $p < 0.05$ in each case).

Treatment with 70 μ M caffeine caused a significant decrease in mean endurance (by 17.6 %) compared to controls when soleus muscle was stimulated maximally at

Table 1 The effects of stimulation frequency on maximum isometric tetanus stress (force normalised to muscle cross-sectional area) and work loop power output (power output normalised to muscle mass) in mouse soleus muscle ($n = 32$ pooled data pre-allocation of preparations to control or caffeine treatment); twitch stress (kN m⁻²) = 41.9 ± 3.9

Stimulation frequency (Hz)	Tetanus stress (kN m ⁻²)*	Max work loop PO (W kg ⁻¹)*
40	150 \pm 17	16 \pm 2
70	188 \pm 17	24 \pm 2
140	202 \pm 11	33 \pm 2

Data represented as mean \pm SE

* Significant differences between stimulation frequencies

140 Hz (Fig. 1; Tukey $p = 0.001$). Mean muscle power output was significantly higher for controls in work loops from 3.6 s until negative work (Fig. 1; two-sample t test, $df = 14$, $p < 0.05$, in each case after measurement at every second loop). Soleus muscle treated with 70 μM caffeine when stimulated submaximally demonstrated a significant mean increase (by 19.2 %) in endurance compared to controls (Fig. 1; Tukey $p < 0.001$). Despite this, no significant difference in mean muscle power output was found between 40 Hz control and 40 Hz caffeine-treated at any individual time point throughout the fatigue run (Fig. 1; two-sample t test, $df = 14$, $p > 0.2$ in each case). An increase in standard error over time represents an increase in individual variation in the rate of fatigue. However, in the 140 Hz caffeine trial, standard error remains low throughout fatigue, thus suggesting that the response to caffeine treatment varies little between individuals.

The effects of 70 μM caffeine on work loop shape

The area of the work loop represents the net work of that particular cycle; the typical work loop shapes presented in Fig. 2 clearly demonstrate that initial net work is larger at 140 Hz stimulation frequency (Fig. 2a) compared to 40 Hz stimulation frequency (Fig. 2c). At 140 Hz stimulation frequency, there is a significant reduction in peak and sustained force through shortening in the caffeine-treated muscle (Fig. 2b) compared to controls (Fig. 2a). The shape of the loop 2.4 s after the start of fatigue indicates that there is an increase in relaxation time that is more pronounced in the caffeine trial (Fig. 2b) compared to controls (Fig. 2a). There is little difference in work loop shapes between controls (Fig. 2c) and those treated with 70 μM caffeine (Fig. 2d) at 40 Hz stimulation frequency. However, the net work throughout fatigue appears to be greater in the caffeine trial compared to controls.

Treatment effects on recovery from fatigue

Soleus muscle fatigued at 140 Hz in control Krebs recovered significantly better than the soleus muscle stimulated at 140 Hz and fatigued in caffeinated Krebs (89 % maximum after 30 min vs. 63 % maximum after 50 min; Tukey $p < 0.001$; Fig. 3). Muscle power output was significantly greater in control treatment at time points 20–60 min (Fig. 3; two-sample t test, $df = 14$, $p > 0.004$ in each case). There was no significant difference in recovery between the control (75 % maximum after 30 min) and those muscles fatigued in caffeine (71 % maximum after 50 min) at 40 Hz (Fig. 3; Tukey $p = 0.795$). Control muscles stimulated throughout at 140 Hz recovered significantly better, relative to pre-fatigue maximum, than those stimulated throughout at 40 Hz (Fig. 3; Tukey $p < 0.001$).

Discussion

The mean untreated maximal isometric tetanic stress and maximal work loop power for the entire population of soleus muscles used in the present study was $202 \pm 11 \text{ kN m}^{-2}$ and $33.1 \pm 2.2 \text{ W kg}^{-1}$, respectively (Table 1), comparable to that previously reported for mouse soleus muscle [3, 8, 28, 32]. Any potential variation in stress and power output between published studies could be attributed to muscle fibre type differences due to variation in strain and age of mice used and the environmental conditions at which they were kept.

The effect of 70 μM caffeine treatment on endurance in maximally stimulated muscle

Incubating soleus muscle in 70 μM caffeine resulted in significantly shorter endurance (by 17.6 %) at maximal (140 Hz) stimulation frequency when compared to controls (Fig. 1). Germinario et al. [34] reported a faster decrease in isolated mouse soleus force when stimulated via repeated tetani after treatment with 2–5 mM caffeine (toxic to humans). The present results contrast those of James et al. [4] who reported that 70 μM caffeine failed to elicit any significant change in the endurance of maximally stimulated EDL. This suggests a fibre type-specific effect of physiologically relevant concentrations of caffeine on muscular fatigue.

In light of our previous findings [5], it is likely that the caffeine treatment in the present study is having a muscle fibre type-specific (when compared with EDL in the previous study by James et al. [4]) and activity level-specific (i.e. only affecting maximally activated muscle) effect on relaxation rate and consequently power output during fatigue. Net power output attained via the work loop technique is the sum of the active work minus the energy required to elongate the muscle back to its original length [36]. If still active at the end of shortening, a greater amount of energy is required to elongate the muscle, as lengthening occurs against a greater resistance. The further elevated increase in relaxation time in caffeine-treated, maximally activated soleus muscle, and the accumulation of this effect over time, is likely to result in a greater degree of muscle activation at the end of shortening. Consequently, in maximally activated muscles, the proportion of negative work per work loop cycle will be greater than in controls causing the muscle to fatigue faster. This is supported via examination of the work loop shapes. The later work loops in the fatigue run suggest that soleus muscle is failing to relax completely before re-lengthening when maximally stimulated, especially when treated with caffeine (Fig. 2, loops 2.4s and 4.8s). The fact that a similar mechanism was not prevalent in EDL [4] highlights the superior calcium handling properties of a faster muscle phenotype.

The effect of 70 μ M caffeine treatment on endurance in submaximally stimulated muscle

Treatment of soleus with 70 μ M caffeine resulted in a mean significant increase in endurance in submaximally stimulated soleus muscle (by 19.2 %) compared to controls (Fig. 1). In contrast to all previous literature [4, 34, 35], the present results are the first to indicate a caffeine-induced direct increase in skeletal muscle endurance. In this instance, it is difficult to make a direct comparison to previous findings primarily due to differences in the method used and the previously high concentrations of caffeine being significantly greater than the physiologically relevant dose [34, 35]. Most previous studies have induced fatigue via repeated isometric tetani; however, this would be a poor predictor of fatigue induced by the patterns of cyclical muscle length changes used in vivo as in isometric studies the muscle is held at constant; therefore force–velocity, length–force and passive properties of the muscle are not considered [29]. The present work is the first to indicate a caffeine-induced, direct increase in skeletal muscle endurance, and further suggests a complex muscle specific caffeine response affected by the intensity of muscle activity.

Examination of the work loop shapes (Fig. 2) indicate that, in contrast to maximally activated muscle, there appears to be no difference in the ability of the muscle to relax in the caffeine-treated muscle (Fig. 2d) when compared to controls (Fig. 2c). As suggested by previous research [4, 5, 20–23], this may indicate an augmented Ca^{2+} release without the effects of amplified negative work observed in maximally fatigued caffeine-treated muscle in the present study. At this reduced frequency of stimulation, a caffeine-induced increase in intramuscular Ca^{2+} is unlikely to exceed the capacity for Ca^{2+} removal at the end of stimulation, thus the effect on relaxation is limited.

Recovery from fatigue following treatment with 70 μ M caffeine

Fatigue of maximally stimulated (140 Hz) soleus muscle treated with caffeine resulted in a significant reduction in recovery (by 26 %) compared to controls (Fig. 3). The previously highlighted caffeine-induced increase in negative work during fatigue at this intensity is the likely cause of this, as elevated eccentric activity is associated with increased muscle damage [36]. Damage brought about by eccentric exercise would result in a decrease in the ability to recover after fatigue, and is an effect previously observed by James et al. [4] using 10 mM caffeine treatment on mouse EDL and soleus. At submaximal, 40 Hz, stimulation frequency, there was no significant difference

between mean recovery of controls and those fatigued in caffeine (Fig. 3).

Practical implications of the present findings

The present findings are the first to investigate the effects of physiological caffeine concentration on the fatigability of predominantly slow twitch muscle, and they demonstrate decreased endurance when maximally stimulated and prolonged endurance when submaximally stimulated. This, in conjunction with our earlier findings in predominantly fast twitch EDL [4], indicates a muscle-specific caffeine fatigue response which is further related to the activation level of the muscle.

Tallis et al. [5] further demonstrated a potentiation in acute muscle power output, independent of stimulation frequency, that was greater in mouse soleus muscle (up to 6 %) compared to EDL (up to 3 %). If applied uniformly across the fatigue data, a 6 % potentiation would result in a slight upwards shift in the fatigue curve, but is unlikely to significantly affect the overall findings.

Although the sinusoidal length change pattern used in the current study provides an approximation of in vivo cyclical muscle activities, it is a simplification of the length change waveforms used in real-life locomotion [37]. In vivo, the pattern of fibre stimulation and length change waveforms are likely to be manipulated throughout movement [38]. Therefore, if a muscle is producing too much eccentric force, the duration of stimulation is likely to be reduced in order to lessen any associated muscle damage. Subsequently, in vivo, the activation of caffeine-treated fibres may be reduced to produce the same power as controls, with the activation of fewer muscle fibres, thus prolonging endurance. However, these proposed differences between our experimental protocols and what may happen in vivo are unlikely to affect the overall findings of this study.

Conclusion

The present work extends our earlier findings [4, 5] by being the first to demonstrate a complex stimulation frequency-dependent response of endurance to physiological concentrations of caffeine. Caffeine treatment caused a significantly faster endurance in maximally stimulated soleus muscle, while, in contrast, endurance was significantly prolonged at a submaximal intensity. The current findings highlight an enhanced ergogenic benefit of caffeine in prolonged submaximal activities that are primarily powered by slower muscle fibres.

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