Effects of caffeine on mouse skeletal muscle power output during recovery from fatigue

Rob. S. James,1 Robbie S. Wilson,2 and Graham N. Askew3

1School of Science and the Environment, Coventry University, Coventry CV1 5FB; 2School of Biology, University of Leeds, Leeds LS2 9JT, United Kingdom; and 3Department of Biology, University of Antwerp, Antwerp B-2610, Belgium

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The effects of 10 mM (high) and 70 μM (physiologically relevant) caffeine on force, work output, and power output of isolated mouse extensor digitorum longus (EDL) and soleus muscles were investigated in vitro during recovery from fatigue at 35°C. To monitor muscle performance during recovery from fatigue, we regularly subjected the muscle to a series of cyclical work loops. Force, work, and power output during shortening were significantly higher after treatment with 10 mM caffeine, probably as a result of increased Ca2+ release from the sarcoplasmic reticulum. However, the work required to relengthen the muscle also increased in the presence of 10 mM caffeine. This was due to a slowing of relaxation and an increase in muscle stiffness. The combination of increased work output during shortening and increased work input during lengthening had different effects on the two muscles. Net power output of mouse soleus muscle decreased as a result of 10 mM caffeine exposure, whereas net power output of the EDL muscle showed a transient, significant increase. Treatment with 70 μM caffeine had no significant effect on force, work, or power output of EDL or soleus muscles, suggesting that the plasma concentrations found when caffeine is used to enhance performance in human athletes might not directly affect the contractile performance of fatigued skeletal muscle.

The effects of peripheral fatigue are mainly due to a combination of impaired Ca2+ release from the sarcoplasmic reticulum (SR) and reduced myofibrillar Ca2+ sensitivity (21). The decreased Ca2+ release during fatigue is linked to a rise in intracellular phosphate ion concentration, which may act by causing precipitation of Ca2+ in the SR (for review, see Ref. 4) and/or inhibition of Ca2+ release from the SR by some other mechanism (9). However, the effects of phosphate appear to be dependent on creatine phosphate concentration, inasmuch as increased phosphate ion concentration in the absence of creatine phosphate causes increased Ca2+ release from the SR (9). Also, decreased ATP concentration and increased adenosine concentration have been linked to a drop in Ca2+ release from the SR (8).

One successful method used to investigate the impairment in Ca2+ release underlying muscle fatigue is an examination of recovery after the introduction of caffeine. In vitro, 3–40 mM (hereafter referred to as millimolar) caffeine has been used to cause rapid but incomplete postfatigue recovery of isometric muscle force generation (3, 19). Caffeine primarily improves muscle force generation by opening the Ca2+ channels in the SR, thereby increasing intracellular Ca2+ concentration (2), but a small proportion of the force increase may also be due to increased Ca2+ sensitivity (3). However, these studies have assessed muscular performance under nonphysiological conditions using high concentrations of caffeine and measuring muscle force responses when the muscle is held at constant length, shortens at constant velocity, or shortens against a constant load (for reviews see Refs. 1 and 10).

During locomotion, many muscles undergo cyclical length changes with phasic activation to produce force and, in some situations, net power output. To maximize net power output during cyclical length changes, the muscle must not only generate high force but also maintain high force during shortening, relax rapidly before subsequent lengthening, and then activate rapidly for high force generation during shortening. The work-loop technique can be used in vitro to subject muscle to cyclical length changes and phasic stimulation (13). Askew and coworkers (6) demonstrated the benefits of using the work-loop technique to investigate the cumulative effects of fatigue (decreased generation and maintenance of force, increased relaxation time, and decreased maximal velocity of shortening) on net power output in mammalian muscle during cyclical length changes. However, no previous study has used the work-loop technique to investigate the possible causes of fatigue in mammalian muscle.

Caffeine has been used by human athletes as an ergogenic aid causing increased endurance and strength performance (for reviews see Refs. 11 and 18). In these reviews, it has been proposed that 10–70 μM (hereafter referred to as micromolar) caffeine in the plasma of athletes provides an ergogenic benefit by altering muscle substrate use during exercise or by affecting the central nervous system to alter motor unit recruitment or by directly affecting intramuscular ion handling (possibly Ca2+ kinetics). It appears that direct effects on the muscle might be from caffeine itself, inasmuch as metabolites of caffeine are unlikely to be in high enough concentrations to mediate an effect (11). However, no previous in vitro study has investigated the direct effect of micromolar concentrations of caffeine on muscle performance.

In this study, we used the work-loop technique to examine the effects of a high concentration of caffeine (10 mM) and a physiologically relevant concentration of caffeine (70 μM) on the ability of isolated mouse skeletal muscles to recover from fatigue.
METHODS

Female mice (bred from the CD1 strain, Charles River) were bred and kept in-house. Eight- to 10-wk-old mice (31.7 ± 3.33 g, n = 37) were killed by cervical dislocation in accordance with the British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1. Although muscle preparations were obtained at two different times from the same mouse colony, body mass was not significantly different between the two groups of mice (P > 0.05).

Soleus and extensor digitorum longus (EDL) muscles were isolated from the hindlimb and pinned out at approximately the muscle’s resting length at room temperature (19–21°C) in oxygenated (95% O2–5% CO2) Krebs-Henseleit solution consisting of (in mM) 118 NaCl, 4.75 KCl, 1.18 MgSO4, 24.8 NaHCO3, 1.18 KH2PO4, 10 glucose, and 2.54 CaCl2, pH 7.60, at room temperature, before oxygenation. EDL and soleus muscles were chosen as examples of muscles of relatively fast- and slow-twitch fiber composition, respectively, although both comprise mixed fiber types (5). The CD1 strain of mice has been found to have fiber type compositions in EDL and soleus muscles similar to those previously reported by Askew and Marsh (5) for the ICR strain of mice (S. A. Jobson and V. M. Cox, unpublished observations). For each muscle, a small piece of bone was left at the end of proximal and distal tendons. Aluminum foil clips were wrapped around the tendons at either end of the muscle, with care taken to leave the sections of bone unwarped to prevent tendon slippage in the clips. Soleus muscles were found to maintain control net power output over the course of an experiment, even if they had been kept at room temperature in a bath of oxygenated Krebs-Henseleit solution for several hours before experimentation. Therefore, both soleus muscles were dissected out from each mouse, and generally both muscles were used for mechanical experiments. However, when two soleus muscles were removed from a mouse, they were never subjected to the same treatment. In contrast, generally only one EDL muscle was used from each mouse.

Isometric studies. The muscle preparation was attached via the foil clips to a force transducer (model UFI, Pidion Controls) at one end and a displacement transducer (model V201, Ling Dynamic Systems) at the other. A linear variable-displacement transformer (model DFG5.0, Solartron Metrology) was used for position detection. The muscle was maintained at 35°C in circulating oxygenated Krebs-Henseleit solution. The preparation was stimulated via parallel platinum electrodes while it was held at constant length to generate a series of isometric Twitches. Stimulus amplitude, pulse width (1.2–1.8 and 1.7 ms for EDL and soleus muscles, respectively), and muscle length were optimized to yield the maximum isometric twitch force. The muscle length that corresponded to maximal isometric twitch production was defined as L0. Mean muscle fiber length was calculated as 75 and 85% of L0 for EDL and soleus muscles, respectively (12), and was 9.36 ± 0.50 (SD) mm (n = 24) and 9.34 ± 0.48 mm (n = 24) for EDL and soleus muscles, respectively. An isometric tetanic contraction was elicited by subjecting the muscle to a train of stimulation (250 and 350 ms for EDL and soleus muscles, respectively). Stimulation frequency was optimized to yield the maximal isometric tetanic force (typically 200–210 and 130 Hz for EDL and soleus muscles, respectively). A recovery period (10 and 5 min for EDL and soleus muscles, respectively) was allowed between each tetanic contraction to ensure that each muscle had fully recovered in its ability to produce maximal force.

Mechanical assessment of muscle performance before fatigue. The work-loop technique (13) was used to determine the power output of muscles during cyclical length changes. The mean muscle length was held at L0 and the muscle stimulation parameters found to be optimal for tetanic contractions were used. Each muscle preparation was subjected to four sinusoidal length change cycles using a total strain of 0.10 mean muscle fiber length given symmetrically around L0 at a cycle frequency of 5 Hz for both muscles. Muscles were stimulated to contract, and the timing and duration of stimulation were optimized to yield the maximum net power output. In addition to active work-loop cycles, muscles were subjected to a series of passive cycles in which the muscles were unstimulated.

Muscle stimulation and length changes were controlled using custom-written software (Testpoint, CEC) via a digital-to-analog board (model KPCI3108, Keithley Instruments). Force and length data were each sampled at a rate of 10 kHz. A plot of force against length produced a “work loop” (13). The work done on the muscle during lengthening (lengthening work; Fig. 1A), the work done by the muscle during shortening (shortening work; Fig. 1B), and the net work (sum of lengthening and shortening work) were determined. The net work generated by the muscle was represented by the area of the work loop, with an anticlockwise work loop indicating net positive work (Fig. 1C) and a clockwise work loop indicating net negative work (Fig. 1D). Net power output of muscle was calculated as net work per cycle frequency. In addition, the peak stress during shortening was determined.

Every 10 min for EDL and every 5 min for soleus, the muscle was subjected to a further set of four work-loop cycles, with stimulation parameters being altered between each set until maximum net work was achieved. For EDL and soleus muscles, a stimulation phase of −5 and −15 ms, respectively (where stimulation phase is calculated as the time when stimulation starts relative to the time when maximum length is reached), and a stimulus burst duration of 100 and 65 ms, respectively, were typically found to yield maximal net power. Before the fatigue run, a set of control sinusoidal length change and stimulation parameters was imposed on the muscle every two to four sets of work loops to monitor variation in the muscle’s ability to generate power. Any variation in power output was assumed to correspond to a change in the ability of the muscle to produce force. This assumption enabled muscle power output between the control runs to be corrected by assuming a linear decline in performance over time.

Induction of fatigue by repeated cyclical length contractions (work loops). After the preliminary optimization of stimulation parameters and assessment of mechanical performance, EDL and soleus muscles were subjected to a fatigue run consisting of 50 and 250 work-loop cycles, respectively. This number of repetitive work-loop cycles resulted in muscle fatigue to the extent that net muscle power had decreased significantly from the control values (P < 0.05).

Fig. 1. Components of a typical mouse extensor digitotum longus (EDL) work-loop cycle optimized for maximal work at 5-Hz cycle frequency. A–C: parts of an active work-loop cycle: A: work done on the muscle during lengthening (lengthening work); B: total work done by the muscle during shortening (positive work); C: net work (positive) produced during length-change cycle (i.e., C = B − A). D: net work done on the muscle during a passive work-loop cycle (negative work). Thickened section of line surrounding the loop in A and C indicates period of electrical stimulation of the muscle. Arrows, direction of work loop (anticlockwise work loops represent net positive work).

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decreased to <25% of the initial value and had reached a plateau (for soleus muscle the plateau was reached at a negative net power value).

Postfatigue treatment and recovery. Immediately after completion of the fatigue run, the bathing solution was replaced with one of the following solutions: 1) standard Krebs-Henseleit solution, 2) Krebs-Henseleit solution containing 10 mM caffeine, or 3) Krebs-Henseleit solution containing 70 μM caffeine. Performance during recovery from fatigue was monitored by regularly subjecting the muscle to a set of four control active or four control passive (no electrical stimulation) work-loop cycles. Preliminary experiments found that soleus muscles recovered more rapidly from fatigue than did EDL muscles; therefore, for soleus muscles a shorter time period was allowed between each set of work loops. After a period (10 and 11 min for soleus and EDL muscles, respectively) in which muscle performance was monitored after fatigue in standard (control) or caffeine (70 μM or 10 mM)-containing bathing solutions, the bathing solution was drained and replaced with standard Krebs-Henseleit solution to allow recovery to be further monitored during a washout period.

Muscle dimension measurements and calculations. Muscle work and power output were expressed relative to muscle mass, which was determined at the end of the experiment using an electronic balance after the muscle was blotted on absorbent paper to remove excess Krebs-Henseleit solution. Muscle stress was calculated as force divided by mean muscle cross-sectional area. Mean muscle cross-sectional area was calculated from mean fiber length, muscle mass, and a muscle density of 1.060 kg/m³ (17).

Statistical analysis. Data obtained during recovery from fatigue were split into two groups: 1) caffeine-treated time points (for active work-loop power output data: 5 and 10 min and 2, 5, 8, and 11 min after the fatigue run for EDL and soleus muscles, respectively) and 2) washout time points (for active work-loop power output data: 15, 20, 30, 40, and 50 min and 14, 17, 20, 23, and 26 min after the fatigue run for EDL and soleus muscles, respectively). The data were analyzed using analysis of variance with Bonferroni’s post hoc test to analyze significant differences in treatment or time. Values represent results from Bonferroni s post hoc tests. Differences between the means were taken to be statistically significant when P < 0.05. Values are means ± SD with the number of replicates (n) indicated in parentheses.

RESULTS

Pretreatment mechanical performance. The mean prefatigue maximum isometric tetanic stress was 267 ± 33.8 (n = 24) and 250 ± 50.1 (n = 24) kN/m² for soleus and EDL muscles, respectively. These results are similar to previously measured values for maximum isometric tetanic stress (5, 12). The mean prefatigue maximum net power output (at 5-Hz cycle frequency and 0.10 strain) was 32.7 ± 4.37 (n = 24) and 52.1 ± 12.3 (n = 24) W/kg for soleus and EDL muscles, respectively. The value for the soleus muscle is similar to previous measurements at 5 Hz: 23 W/kg (12) and 44 W/kg (5). However, the value for the EDL muscle is lower than has previously been reported: 85 W/kg (12) and 121 W/kg (5). This may reflect differences in experimental procedures [e.g., Askew and Marsh (5) optimized strain in their experiments] or differences in the strain of mice used. It is unlikely to quantitatively affect the conclusions of our experiments.

Fatigue resulted in a decline in net muscle power output to a mean of ~8.51 W/kg in soleus muscle (after 250 work loops; Fig. 2A) and 6.28 W/kg in EDL muscle (after 50 work loops; Fig. 2B). The negative power output of fatigued soleus muscle indicates that work must be performed on the muscle for it to undergo active cycling. For EDL and soleus muscles, there was no significant difference (P > 0.05) in net power output between the three treatment groups at the end of the fatigue period, i.e., just before treatment commenced.

Effects of caffeine concentration on power output. The control group power output for soleus and EDL muscles showed almost complete recovery from fatigue, attaining means of 87 and 100%, respectively, of the prefatigue values (Fig. 2). Recovery of the EDL muscle took more than twice as long as recovery of the soleus muscle. The pattern of recovery exhibited by both muscles that were bathed in Krebs-Henseleit solution containing 70 μM caffeine after the fatigue run was indistinguishable (P > 0.05) from that exhibited by the control group at all stages of the treatment and washout periods (Fig. 2).

The power output of soleus muscles that were bathed in Krebs Henseleit solution containing 10 mM caffeine after the fatigue run continued to decline throughout the treatment period. Power output was significantly lower in soleus muscle treated with 10 mM caffeine than in control or preparations treated with 70 μM caffeine throughout the treatment period (Fig. 2A; P < 0.01 in each case). The decrease in the power output of the soleus muscle over time during 10 mM caffeine treatment was significant when post hoc tests compared 2 min with 11 min after fatigue (P = 0.01). During the washout period after 10 mM caffeine treatment, the power output increased, reaching a plateau of ~60% of the prefatigue power output. In contrast, EDL muscles that were bathed in Krebs-Henseleit solution containing 10 mM caffeine initially showed an improvement of power output to ~90% of prefatigue power output (Fig. 2B). This increased power output was not significantly different from that with other treatments. However, inasmuch as the prefatigue power output was lower for EDL muscle treated with 10 mM caffeine than for control muscle (Fig. 2B), when the recovery data were expressed as a percentage of the prefatigue power output, 5 min of treatment with 10 mM caffeine yielded significantly higher values than controls (P < 0.05; Fig. 2C). After the initial increase in postfatigue power output of EDL muscle, power output decreased throughout the latter part of the treatment period, and the decline continued into the early part of the washout period. Power output started to increase during the latter part of the washout period and recovered to a mean value in excess of 90% of prefatigue values 50 min after the fatigue run.

Effects of caffeine concentration on peak work-loop stress. Peak work-loop stress (LSmax) was significantly reduced after the fatigue run for soleus and EDL muscles (Fig. 3) to mean values of 45.9 and 50.1%, respectively (considering all treatment groups together). Control group LSmax increased during the recovery period to ~60 and 81% of the prefatigue values for soleus and EDL muscles, respectively (Fig. 3). There was no significant difference between LSmax developed by the control and 70 μM caffeine-treated muscles in the treatment and washout stages of recovery (P > 0.05).

LSmax was significantly higher in 10 mM caffeine-treated soleus and EDL muscles during the treatment phase than in control or 70 μM caffeine-treated muscles (Fig. 3; P < 0.01 in each case). LSmax significantly exceeded prefatigue values for the soleus muscle during the treatment phase (P < 0.01). However, for both muscles, LSmax declined during the washout period, reaching values that were not significantly different from control or 70 μM caffeine-treated muscles (Fig. 3).
Effects of caffeine concentration on power generated during lengthening and shortening in active and passive cycles. The net power generated during cyclical contractions during recovery was also expressed as two separate components: the power generated during shortening (POW\text{short}) and the power absorbed during lengthening (POW\text{len}). POW\text{len} for control soleus muscles became more negative during the fatigue run but subsequently gradually recovered to prefatigue values (Fig.

Fig. 2. Net muscle power output during recovery from fatigue for control and caffeine-treated soleus (A) and EDL (B and C) muscles. Pre-time 0, prefatigue; time 0, end of fatigue run; dashed vertical lines, start and end of treatment (Treat) period. Values are means ± SD; \( n = 8 \) for each treatment for each muscle. □, Control muscles; ◊, 70 μM caffeine-treated muscles; ●, 10 mM caffeine-treated muscles. In A and B, power output is normalized relative to muscle mass; in C, power output of EDL is expressed as a percentage of prefatigue values. *\( P < 0.05 \), 10 mM caffeine vs. control and 10 mM vs. 70 μM. §\( P < 0.05 \), 10 mM vs. control.

Fig. 3. Peak work-loop stress during recovery from fatigue for control and caffeine-treated soleus (A) and EDL (B) muscles. Pre-time 0, prefatigue; time 0, end of fatigue run; dashed vertical lines, start and end of treatment period. Force is normalized to cross-sectional area (i.e., stress). Values are means ± SD; \( n = 8 \) for each treatment for each muscle. □, Control muscles; ◊, 70 μM caffeine-treated muscles; ●, 10 mM caffeine-treated muscles. *\( P < 0.05 \), 10 mM vs. control and 10 mM vs. 70 μM.
POW_short was depressed after fatigue for control soleus and EDL muscles but recovered to ∼90–95% of prefatigue values (Fig. 4, C and D). The time course and magnitude of alterations in POW_short and POW_len were not significantly different for 70 μM caffeine-treated soleus and EDL muscles and control muscles.

Treatment with 10 mM caffeine caused POW_len for soleus and EDL muscles to become markedly more negative (Fig. 4, A and B). POW_len for soleus and EDL muscles was significantly more negative in 10 mM caffeine-treated muscles than in control groups throughout the period of treatment (Fig. 4, A and B; P < 0.05). POW_short was significantly higher for the first two 10 mM caffeine-treated time points for EDL and soleus muscles than for any other treatments before the washout (Fig. 4, C and D; P < 0.05). During the washout period, POW_short and POW_len recovered to approximately their prefatigue values for soleus and EDL muscles.

Passive power output (POW_pass) was unaffected by the fatigue protocol in control and 70 μM caffeine-treated soleus and EDL muscles. However, POW_pass became more negative in 10 mM caffeine-treated soleus and EDL muscles toward the end of the treatment period and early into the washout phase, where there was a significant difference from control muscles (P < 0.01 at 17.5 min for EDL muscle and 15.5 and 21.5 min for soleus muscle). There was no significant effect of treatment on POW_pass before the washout phase (Fig. 5). These changes in POW_pass were accompanied by an increase in muscle stiffness during stretch, evidenced by an increase in the slope of the stress-strain plot during lengthening (Fig. 6).

DISCUSSION

Effects of 10 mM caffeine on recovery from fatigue. In the present study, fatigued soleus and EDL muscles treated with 10

![Fig. 4. Power output during lengthening and shortening phases of cyclical contractions during recovery of mouse soleus (A and C) and EDL (B and D) muscles from fatigue. Pre-time 0, prefatigue; time 0, end of fatigue run; dashed vertical lines, start and end of treatment period. Power output is normalized to muscle mass. Values are means ± SD; n = 8 for each treatment for each muscle. □, Control muscles; △, 70 μM caffeine-treated muscles; ●, 10 mM caffeine-treated muscles. *P < 0.05, 10 mM vs. control and 10 mM vs. 70 μM. §P < 0.05, 10 mM vs. control.](http://jap.physiology.org/)}
mM caffeine generated higher peak stress during cyclical contractions than control muscles or muscles treated with 70 μM caffeine (Fig. 3), and for the soleus muscle, peak stress was increased to levels greater than in unfatigued muscle. Muscles also generated higher power output during shortening than control and 70 μM caffeine-treated muscles (Fig. 4, C and D) as a result of increased force generation during this phase of the cycle. Development of peripheral fatigue in muscle is believed to primarily be due to impaired cross-bridge cycling due to reduced Ca\(^2+\)/sensitivity of the myofilaments, reduced cross-bridge force production, and impaired SR Ca\(^2+\) release (for review see Refs. 1 and 4). Previous studies have shown that application of 10 mM caffeine to fatigued muscle can restore isometric tetanic tension to \(\approx 80\%\) of prefatigue values by facilitating SR Ca\(^2+\) channel opening (21). Thus our studies have shown that in cyclical contractions, as in isometric tetanic contractions, millimolar concentrations of caffeine are generally able to restore contractile function.

Net power output in soleus muscle after treatment with 10 mM caffeine was actually lower than control values, despite the increase in force and work output during shortening. In the EDL muscle, net power output was higher than in control muscles at the start of the treatment period (significantly higher when expressed as a percentage of prefatigue values). However, for both muscles, net power output decreased during the treatment period (Fig. 2). The reduction in net power output in the soleus muscle is attributable to an increase in the work required to reextend the muscle (see control muscle in Fig. 3) (6). In the presence of 10 mM caffeine, the work that must be performed on the soleus and EDL muscles during lengthening is significantly higher than in the control muscles (Fig. 4, A and B) and, presumably, results from a combination of enhanced Ca\(^2+\) release due to the caffeine and impaired Ca\(^2+\) reuptake due to the effects of fatigue. 3) The increase in lengthening work may have been due to fiber damage caused by treatment of the muscle with a millimolar concentration of caffeine (14). However, this seems to have been minimal based on histological examination of the muscles (14).
unlikely, because, during washout, work-loop stress did recover to control values (Fig. 3). In contrast to the soleus muscle, the EDL muscle exhibited increased power output compared with control muscles at the onset of the treatment period. This may be due to the cycle frequency at which the experiments were performed or differences in fiber type. The optimum cycle frequency for maximum net power output is 3–5 Hz for the soleus muscle and 9–12 Hz for the EDL muscle (5, 12). Therefore, the cycle frequency used in these experiments (5 Hz) is likely to be close to the optimum for power generation in soleus muscle but much lower than the optimum in EDL muscle. Relaxation represents a much greater proportion of the cycle in the soleus muscle at 5 Hz than in the EDL muscle (see Fig. 1 in Ref. 12). Changes in the duration of relaxation brought about by fatigue and treatment with caffeine that impair muscle net power output are therefore likely to have a greater effect in the soleus than in the EDL muscle. Further studies optimizing cycle frequency in the EDL muscle would help determine whether the differences were genuine fiber type effects or merely a consequence of the cycle frequency used in the present study.

Effects of 70 µM caffeine. In the present in vitro study, 70 µM caffeine had no significant effect on net power output or passive power of soleus and EDL muscles during recovery, and the results were indistinguishable from those in fatigued control muscles. Previous in vivo studies on human performance have suggested that micromolar caffeine concentrations have direct intramuscular effects, causing improved locomotory performance by increased sodium-potassium pump activity (15) or by direct action on the ryanodine receptor to enhance Ca^{2+} release (for review see Ref. 20). In light of our results, this suggests that 1) the effect of 70 µM caffeine applied to nonfatigued muscle is substantially different from the effect of 70 µM caffeine applied to fatigued muscle or 2) the concentrations of caffeine found in human athletes are unlikely to directly affect skeletal muscle performance. It may be that any in vivo effect of caffeine ingestion on skeletal muscle performance is an indirect effect due to the subsequent increase in epinephrine affecting muscle metabolism (7) or due to direct effects on the central nervous system altering perceived exertion or recruitment of motor units (for reviews see Refs. 11 and 18).

Relevance of the present study to locomotion. EDL and soleus muscles have been used in this study as examples of typical fast- and slow-twitch muscles. They were not chosen for this study because they are likely to undergo sinusoidal length trajectories in vivo. During locomotion in the mouse, the soleus muscle is thought to be active during shortening and isometric phases of movement, whereas the EDL muscle is typically active during shortening (12). During locomotion, the timing and duration of fiber recruitment and the pattern of lengthening and shortening are likely to change as a mechanism to delay muscle fatigue (6). Length change waveforms used by these muscles in vivo are more complex than the sinusoidal waveforms used in this study (12), and waveform complexity can have large effects on muscle force and power output (for review see Ref. 16). However, these experiments were designed to explore the effects of caffeine on work and stress during recovery from fatigue using two well-characterized muscles performing cyclical contractions.

Conclusion. The depressant effects of fatigue on force and power output during shortening are reversed in soleus and EDL muscles by exposure to 10 mM caffeine. However, the increased power required to lengthen the muscle, due to increased muscle stiffness and slowing of relaxation, resulted in decreased net power output of soleus muscle and only a short-lived increase in the net power output of EDL muscle. Concentrations of caffeine that have been shown to result in improved performance in vivo (70 µM) were found to have no significant effect in vitro, suggesting that the effects seen in human athletes may be due to the indirect effects of caffeine.

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