The effect of physiological concentrations of caffeine on the power output of maximally and submaximally stimulated mouse EDL (fast) and soleus (slow) muscle

Jason Tallis, Rob S. James, Val M. Cox, and Michael J. Duncan
Faculty of Health and Life Sciences, Coventry University, Coventry, United Kingdom
Submitted 28 June 2011; accepted in final form 3 October 2011

Tallis J, James RS, Cox VM, Duncan MJ. The effect of physiological concentrations of caffeine on the power output of maximally and submaximally stimulated mouse EDL (fast) and soleus (slow) muscle. J Appl Physiol 112: 64–71, 2012. First published October 6, 2011; doi:10.1152/japplphysiol.00801.2011.—The ergogenic effects of caffeine in human exercise have been shown to improve endurance and anaerobic exercise performance. Previous work has demonstrated that 70 μM caffeine (physiological maximum) can directly increase mouse extensor digitorum longus (EDL) muscle power output (PO) in sprintlike activity by 3%. Our study used the work loop technique on isolated mouse muscles to investigate whether the direct effect of 70 μM caffeine on PO differed between 1) maximally and submaximally activated muscle; 2) relatively fast (EDL) and relatively slow (soleus) muscles; and 3) caffeine concentrations. Caffeine treatment of 70 μM resulted in significant improvements in PO in maximally and submaximally activated EDL and soleus (P < 0.03 in all cases). For EDL, the effects of caffeine were greatest when the lowest, submaximal stimulation frequency was used (P < 0.001). Caffeine treatments of 140, 70, and 50 μM resulted in significant improvements in acute PO for both maximally activated EDL (3%) and soleus (6%) (P < 0.023 in all cases); however, there was no significant difference in effect between these concentrations (P > 0.420 in all cases). Therefore, the ergogenic effects of caffeine on PO were higher in muscles with a slower fiber type (P < 0.001). Treatment with 35 μM caffeine failed to elicit any improvement in PO in either muscle (P > 0.72 in both cases). Caffeine concentrations below the physiological maximum can directly potentiate skeletal muscle PO. This caffeine-induced increase in PO could provide similar benefit across a range of exercise intensities, with greater gains likely in activities powered by slower muscle fiber type.

CAFFEINE (COMMON NAME FOR 1,3,7-trimethylxanthine) is a powerful ergogenic aid that has been extensively studied for its effects in improving exercise capacity (13). In vivo and in vitro studies have found enhancements in endurance exercise performance, power, and fatigue recovery, accredited primarily to the effects of caffeine on the central nervous system (20). Despite its documented popularity as a performance enhancer, aiding training and competition, caffeine presently still falls short of the World Anti-Doping Agency’s prohibited list (35). Reviews by Graham (13) and Davis and Green (8) suggest that caffeine can enhance performance during endurance (activity lasting >30 min), power, and strength activities.

Following digestion, caffeine can be readily absorbed into the bloodstream, with peak plasma concentration occurring 30–60 min after ingestion (21). Its hydrophobic nature allows free passage of caffeine across all biological membranes, resulting in distribution throughout all of the tissues of the body (23). Caffeine is also able to diffuse from the cerebral circulation across the blood-brain barrier, entering the cerebrospinal fluid in sufficient quantity to promote pharmacological effects (10, 12, 24, 33). The primary action of caffeine is to act centrally as a competitive adenosine receptor antagonist, increasing transmission via dopamine D2 receptors (10, 28). Lorist and Tops (21) explored behavioral and performance responses to caffeine ingestion, demonstrating an increase in response to stimuli, an elevated state of arousal, and a decreased rate of perceived exertion. Caffeine has also been demonstrated to have a direct effect on skeletal muscle by acting as an adenosine receptor antagonist on A1 receptors directly on the skeletal muscle membrane and/or by binding to the ryanodine receptors (RyR) of the sarcoplasmic reticulum (SR), resulting in altered excitation contraction coupling (4, 7, 10, 27).

Early in vitro studies demonstrated the direct potentiating effects of caffeine on acute muscle twitch and tetanus force; however, many of these studies used supraphysiological, millimolar concentrations of caffeine, which would be toxic to humans (9, 10, 14, 22, 32). The primary mechanism by which caffeine can promote enhanced force output in skeletal muscle is believed to be via interference of excitation contraction coupling (8). It has been established that the specific mechanism of action is alteration of intramuscular ion handling, primarily via an increased concentration of Ca2+ within the intracellular space (23). However, little is known about whether variation in physiological conditions, such as intensity of exercise and caffeine dosage, will alter the direct response of muscle to caffeine during human physical activities. Tarnopolsky and Cupido (29) reported that 6 mg/kg body mass (~60 μM in blood plasma) of caffeine enhanced involuntary evoked skeletal muscle force in human subjects at low, but not high, stimulation frequencies. This was attributed to a potentiation of calcium release at lower stimulation frequencies, promoting a greater influx of Ca2+ in the presence of caffeine. However, no previous in vitro study has directly tested isolated muscle to determine whether the enhancement of force and power production in skeletal muscle, due to caffeine treatment, is greater at lower stimulation frequencies. Such findings would be of practical benefit to athletes, as they would indicate the types of physical activities in which the ergogenic effects of caffeine were greatest.

James et al. (16, 17) were the first to test the effect of physiologically relevant concentrations of caffeine (70 μM human in vivo maximum, Ref. 13) using the work loop technique. They found a small but significant, 2–3%, increase...
in mean net power output (PO) in maximally activated isolated mouse extensor digitorum longus (EDL; fast muscle), attributed to increased force production during shortening. Caffeine treatments of 70 μM had no significant effect on delaying the onset of fatigue or enhancing fatigue recovery. Evidence from use of millimolar concentrations of caffeine (which would represent toxic blood plasma concentrations in humans, Ref. 10) has shown that potentiation occurs to a greater extent in relatively slower muscle e.g., soleus (11, 27, 34). This has largely been accredited to differences in Ca\(^{2+}\) kinetic properties (23). However, no previous study has tested whether there is a difference between muscle fiber types in the direct effect of physiological concentrations of caffeine on PO. A dose-dependent effect on direct muscle performance has further been demonstrated with high 0.07–20 mM concentrations of caffeine (11, 16); however, this response has not been investigated over physiologically relevant caffeine concentrations; therefore, there are currently no studies to indicate the dosage of caffeine required for humans to maximize PO in muscle during physical activity.

The present study aims to investigate whether maximal physiological concentrations (70 μM) of caffeine directly affects the PO of isolated skeletal muscle during brief bouts of cyclical activity, being the first such study to compare between (1) maximally and submaximally activated muscle; 2) relatively fast EDL and relatively slow soleus muscles; and 3) micromolar concentrations (35–140 μM) of caffeine.

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) were bred and kept at Coventry University. Eight- to ten-week-old mice (body mass = 30.2 ± 0.8 g; mean ± SE, n = 108) were weighed and then killed by cervical dislocation, in accordance with British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1.

Soleus or EDL muscle was isolated from the right hindlimb and 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 the following composition (mM): 118 NaCl; 4.75 KCl; 1.18 MgSO\(_4\); 24.8 NaHCO\(_3\); 1.18 KHPO\(_4\); 10 glucose; 2.54 CaCl\(_2\); pH 7.55, at room temperature before oxygenation. For each preparation, the tendon and a small piece of bone was left attached at the proximal and distal ends. Aluminum foil T-clips were wrapped around each tendon, leaving the bone at the back of the clip to help minimize tendon slippage when the muscle was producing force (16).

Isometric studies. Foil clips were used to attach the muscle preparation via crocodile clips, at one end to a force transducer (UF1, Pioden Controls), and at the opposing end to a motor (V201, Ling Dynamic Systems). Position of the motor arm was detected via a Linear Variable Displacement Transformer (DFGS5.0, Solartron Metrology).

The muscle was maintained in circulated oxygenated Krebs-Henseleit solution at a constant temperature of 36 ± 0.36°C. The preparation was stimulated 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 fiber itself, but stimulated the muscle via the surrounding fluid.

Muscle length and stimulus amplitude (12–16 V for soleus; 14–18 V for EDL) were optimized 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\(_m\). Mean muscle fiber length was calculated as 85% of L\(_m\) (15). Maximal isometric tetanic force was measured by subjecting the preparation to a burst of electrical stimuli (320 ms for soleus; 200 ms for EDL). Stimulation frequency was optimized to yield maximal tetanic force (normally 140 Hz for soleus; 200 Hz for EDL), following this further tetanic responses were measured at two submaximal stimulation frequencies (70 and 40 Hz for soleus; 150 and 100 Hz for EDL). A 5-min rest period was imposed between each tetanus to ensure the muscle had sufficient recovery time.

The same isometric protocol was used for all EDL and soleus preparations before beginning the work loop experiments to determine the acute effects of caffeine.

Work loop studies. The work loop technique assesses the ability of the muscle to produce power while undergoing cyclical length changes (16, 18, 19). Here the muscle was held at L\(_m\), and the stimulation amplitude and 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\(_m\), followed by a shortening to 5% shorter than L\(_m\), before returning back to L\(_m\), at a cycle frequency of 5 Hz. Five-Hertz cycle frequency was used for soleus preparations as it represents the cycle frequency that has previously been shown to elicit maximal PO in mouse soleus muscle and is attainable in running mice (3, 15). Five Hertz was also used for EDL preparations to enable a direct comparison with soleus muscles; however, maximal PO for EDL is achieved at 10-Hz cycle frequency (15). The strain used comes from previous estimation of strains that produce maximal PO at 5 Hz in soleus and EDL and that are attainable during in vivo locomotion (15, 30). Muscle stimulation and length changes were controlled using custom-written software (Testpoint, CEC) via a D/A board (KPCI3108, Keithley Instruments). 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 represents the net work done by the muscle during a single length-change cycle (19). The preparations were electrically stimulated by altering burst duration until maximal net PO was achieved.

A burst duration of 100 ms was found to elicit maximal PO in EDL, consistent with the findings of James et al. (16, 17). The burst duration dictates the number of stimuli that the muscle receives during the work loop; optimizing this duration maximizes PO. Usually a burst duration of 65 ms was found to elicit maximal PO in soleus, consistent with the findings of James et al. (16, 17) and Vassilakos et al. (30). However, on occasions when subjecting soleus to a 40-Hz stimulation frequency, the burst duration was lengthened to 76 ms, adding a further stimulus during the shortening phase of the work loop. This adjustment was determined by examining PO values. If the muscle is too active during lengthening, there is greater resistance to elongate the muscle back to resting length, and therefore a decreased net PO. A stimulation phase shift of −10 ms was fixed for all preparations in the present study (30). The stimulation phase shift dictates that stimulation of the muscle starts 10 ms before the muscle reaches maximal length; therefore, with a stimulus duration of 65 ms in soleus, stimulation continues until 45 ms before the muscle reaches its shortest length.

Before commencement of testing, muscle PO was measured at maximal and submaximal stimulation frequencies in all of the preparations used (140, 70, and 40 Hz for soleus; 200, 150, and 100 Hz for EDL). The second loop of each set of four work loops was used as an indicative measure for each trial, as it did not prove to be different from loop 3. Following this, all of the length and stimulation parameters were kept constant, and a 10-min rest between each trial was enforced to allow maximal recovery time (17).

Muscle preparations were subjected to four work loops at 10-min intervals over a 120-min duration. The protocol consisted of three control measurements in standard Krebs-Henseleit solution, followed
by six measurements in Krebs-Henseleit solution containing 70 \( \mu \)M caffeine, concluding with a washout period of four measurements in standard Krebs-Henseleit solution. To test for a possible interaction between caffeine and stimulation frequency, this procedure was repeated using 140-, 70-, or 40-Hz and 200-, 150-, or 100-Hz stimulation frequencies for soleus and EDL, respectively (\( n = 8 \) in all cases). To examine the effects of altered caffeine concentration, the same procedure was followed; however, the concentration of caffeine added to the Krebs-Henseleit solution was altered to 140, 50, or 35 \( \mu \)M.

Muscle mass measurements and dimension calculations. At the end of the experiment, the tendons were 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 to the nearest 0.0001 g. Mean muscle cross-sectional area was calculated from mean fiber length, muscle mass, and an assumed muscle density of 1,060 kg/m\(^3\) (25). Isometric stress was calculated as force divided by mean muscle cross-sectional area. Muscle PO was normalized to muscle mass to express power as Watts per kilogram.

Statistical analysis of the data. Single-factor ANOVAs were performed in SPSS (version 16, SPSS) to investigate the difference in isometric stress and work loop power between EDL and soleus muscles. Further single-factor ANOVAs were performed to examine the effect of stimulation frequency, before any caffeine treatment, on isometric stress in soleus, isometric stress in EDL, work loop power in soleus, and work loop power in EDL.

Before commencement of testing, muscle stress and PO at 140, 70, and 40 Hz for soleus and 200, 150, and 100 Hz for EDL were measured in all of the preparations used. Two-factor ANOVAs (2 \( \times \) 3 ANOVAs) were conducted on this data to test for significant differences between stimulation frequencies and the caffeine treatment categories in which the preparations were subsequently placed. Therefore, stimulation frequency and caffeine treatment category were used as the fixed factors and PO as the dependent variable. Tukey post hoc tests were performed for stimulation frequency where any significant differences were found.

Before testing the effect of 70 \( \mu \)M caffeine over different stimulation frequencies, there was no significant difference in stress and PO between caffeine treatment categories in soleus and EDL (ANOVA \( P < 0.65 \) in all cases) before caffeine treatment. Before testing the effects of different caffeine concentrations, there was no significant difference in stress and PO between treatment categories in EDL (ANOVA \( P = 0.723 \)). In soleus, the 50 \( \mu \)M treatment group produced significantly more stress than the 35 \( \mu \)M group (ANOVA Tukey \( P < 0.001 \)); however, there was no significant difference between any of the other treatment groups (Tukey \( P > 0.505 \) in all cases). For soleus and EDL, there was no significant difference in PO between the treatment categories (\( P = 0.695 \) in both cases). A reduction in isometric stress resulted in a reduction in stress and power in all treatment groups for both EDL and soleus (ANOVA \( P < 0.001 \) in all cases). Therefore, it is fair to conclude the preparations were of similar quality before treatment.

Muscle PO will decrease over time due to the gradual development of an anoxic core. To avoid deterioration in muscle performance masking the effects of caffeine, a first-order regression equation was calculated using the control data and washout data to identify the linear relationship between muscle PO and time. This regression equation was then used to determine theoretical control muscle PO for each time point during caffeine treatment. The range of regression coefficients were \( R^2 = 0.002–0.9972 \); the level of significance between these regressions varied between \( P < 0.001 \) and 0.883. Typically muscle preparations that demonstrated a degree of deterioration over time showed significant regression coefficients normally exceeding 0.8 (\( P < 0.05 \) in each case). Preparations that were stable over time had low regression coefficients, and the effect of the correction was minimal.

A single-factor ANOVA was conducted on each treatment group to determine any difference between prior treatment control and post-treatment washout. For soleus and EDL muscles, there was no significant difference between the prior and post treatment controls. Therefore, it is assumed that, after the caffeine treatment, the muscles returned to their previous state, and any changes in performance during treatment were solely the effects of caffeine. These control data were pooled, and subsequent analysis was conducted comparing caffeine treatment directly against controls.

The effects of stimulation frequency (100, 150, and 200 Hz) and caffeine treatment (70 \( \mu \)M caffeine or control) and caffeine concentration as the fixed factors. A significant interaction between concentration and treatment was identified in EDL treated, with 70 \( \mu \)M caffeine at different stimulation frequencies and both soleus and EDL treated with altered caffeine concentrations [two factor (2 \( \times \) 3) ANOVA, \( P < 0.015 \) in all cases]; therefore, we conducted a single-factor ANOVA on each treatment group to determine the effect of caffeine compared with control.

Results were interpreted as significant when \( P < 0.05 \). Values are displayed as means \( \pm \) SE.

RESULTS

EDL produced significantly greater stress and greater PO than soleus (Table 1; single-factor ANOVA main effect \( P < 0.001 \) in both cases). Reducing stimulation frequency resulted in a significant reduction in stress (to 87.8 and 66.4% of maximal for soleus when stimulated at 70 and 40 Hz and to 91.1 and 69.6% of maximum for EDL when stimulated at 150 and 100 Hz, respectively) for both soleus and EDL [Table 1; two-factor (2 \( \times \) 3) ANOVA main effect, \( P < 0.001 \) in both cases]. A reduction in stimulation frequency also resulted in a significant decrease in maximum work loop PO (to 64.3 and 42.9% of maximum for soleus when stimulated at 70 and 40 Hz and to 87.1 and 59.5% of maximum for EDL when stimulated at 150 and 100 Hz, respectively) in soleus and EDL [Table 1; two-factor (2 \( \times \) 3) ANOVA main effect, \( P < 0.001 \) in both cases].

<table>
<thead>
<tr>
<th>Table 1. Mean effect of altered stimulation frequency on tetanus stress and work loop power in mouse EDL and soleus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soleus Stimulation Frequency</strong></td>
</tr>
<tr>
<td>40 Hz</td>
</tr>
<tr>
<td>Twitch stress, kN/m(^2)</td>
</tr>
<tr>
<td>Tetanus stress, kN/m(^2)</td>
</tr>
<tr>
<td>Maximum work loop PO, W/kg</td>
</tr>
<tr>
<td>70 Hz</td>
</tr>
<tr>
<td>Twitch stress, kN/m(^2)</td>
</tr>
<tr>
<td>Tetanus stress, kN/m(^2)</td>
</tr>
<tr>
<td>Maximum work loop PO, W/kg</td>
</tr>
<tr>
<td>140 Hz</td>
</tr>
<tr>
<td>Twitch stress, kN/m(^2)</td>
</tr>
<tr>
<td>Tetanus stress, kN/m(^2)</td>
</tr>
<tr>
<td>Maximum work loop PO, W/kg</td>
</tr>
<tr>
<td>100 Hz</td>
</tr>
<tr>
<td>Twitch stress, kN/m(^2)</td>
</tr>
<tr>
<td>Tetanus stress, kN/m(^2)</td>
</tr>
<tr>
<td>Maximum work loop PO, W/kg</td>
</tr>
<tr>
<td>150 Hz</td>
</tr>
<tr>
<td>Twitch stress, kN/m(^2)</td>
</tr>
<tr>
<td>Tetanus stress, kN/m(^2)</td>
</tr>
<tr>
<td>Maximum work loop PO, W/kg</td>
</tr>
<tr>
<td>200 Hz</td>
</tr>
<tr>
<td>Twitch stress, kN/m(^2)</td>
</tr>
<tr>
<td>Tetanus stress, kN/m(^2)</td>
</tr>
<tr>
<td>Maximum work loop PO, W/kg</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE. EDL, extensor digitorum longus; PO, power output.
Effects of stimulation frequency and 70 μM caffeine treatment on muscle PO. Caffeine treatment (70 μM) of soleus elicited significantly greater PO than that of controls in all cases (Fig. 1; single-factor ANOVA main effect, \( P < 0.005 \) in all cases). Caffeine elicited a mean increase in peak PO of 6.4, 6.2, and 5.7% for 140-, 70-, and 40-Hz stimulation frequencies, respectively. There was no significant difference in the effect of caffeine between stimulation frequencies [Fig. 1; two-factor (2 x 3) ANOVA main effect, \( P = 0.093 \)]. Caffeine treatment (70 μM) of EDL elicited significantly greater PO than that of controls (Fig. 2; single-factor ANOVA main effect, \( P < 0.005 \) in all cases). Caffeine elicited a mean increase in peak PO of 3.3, 4.2, and 6.7% for 200-, 150-, and 100-Hz stimulation frequencies, respectively. There was no significant difference in the effect of 70 μM caffeine between 200 and 150 Hz (Fig. 2; Tukey \( P = 0.976 \)). However, 70 μM caffeine treatment elicited a significantly greater increase in PO at 100 Hz compared with 200 and 150 Hz [Fig. 2; two-factor (2 x 3) ANOVA Tukey, \( P < 0.005 \) in both cases].

A set of responders and a set of nonresponders (those showing no noticeable improvement in response to treatment) were evident in soleus (Fig. 3) and EDL (Fig. 4) in all of the treatment groups besides 100-Hz submaximally stimulated EDL. Caffeine-treated EDL, stimulated at 100 Hz, showed no obvious nonresponders to the treatment (Fig. 4C).

The effect of 35, 50, 70, and 140 μM caffeine treatment on muscle PO. Treatment of soleus muscle with 140, 70, and 50 μM caffeine resulted in a significant increase in maximal power of up to 6% (Fig. 5, single-factor ANOVA main effect, \( P < 0.015 \) in all cases). Treatment using 35 μM caffeine failed to significantly increase soleus muscle’s maximal PO (Fig. 5, single-factor ANOVA main effect, \( P = 0.072 \)). There was no significant difference in the increase in PO between 140, 70, and 50 μM caffeine treatments [Fig. 5; two-factor (2 x 3) ANOVA Tukey, \( P < 0.473 \) in all cases]. Treatment of EDL muscle with 140, 70, and 50 μM caffeine resulted in a significant increase in mean maximal power of up to 3.3% (Fig. 6; single-factor ANOVA main effect, \( P < 0.022 \) in all cases). Treatment using 35 μM caffeine failed to significantly increase EDL muscle maximal PO (Fig. 6; single-factor ANOVA main effect, \( P = 0.341 \)). There was no significant difference in the increase in PO between 140, 70, and 50 μM caffeine treatments [Fig. 6; two-factor (2 x 3) ANOVA Tukey, \( P > 0.421 \) in all cases].

As there was no significant difference in response between 140, 70, and 50 μM caffeine treatments, these results were pooled, and soleus was compared against EDL. Treatment of soleus muscles with 50–140 μM caffeine resulted in a significantly greater increase in PO (4.7%) compared with EDL (2.5%) muscle [Fig. 7; ANOVA two-factor (2 x 3) main effect, \( P < 0.001 \)].

**DISCUSSION**

The mean maximal isometric tetanic stress was 189 ± 12 and 300 ± 23 kN/m² for soleus and EDL, respectively (Table 1). This is similar to soleus, but notably higher for EDL stresses previously reported by James et al. (15, 17) and Vassilakos et al. (30) in studies using similar methods. The mean untreated maximal PO was 31.7 ± 1.8 and 85.2 ± 7.1 W/kg (Table 1), again, similar to the values reported by James et al. (15, 17), Askew et al. (3), and Vassilakos et al. (30). Any differences in stress and PO between studies could be attributed to muscle fiber-type differences due to variation in strain and age of the mice and the environmental conditions at which they were kept. Variation in muscle mass and length will also affect the maximal stress and power that the muscle can achieve.

The effects of 70 μM caffeine on muscle PO at maximal and submaximal stimulation frequencies. Treatment of mouse EDL and soleus muscle with 70 μM caffeine elicited significantly greater PO. A mean increase in soleus PO of ~6% occurred at each stimulation frequency (Fig. 1). In EDL, the caffeine-induced enhancement of PO decreased with increased stimulation frequency from 6.7% at 100 Hz to 3.3% at 200 Hz (Fig. 2). The ergogenic benefit was not significantly different between stimulation frequencies in soleus; however, a lower stimulation frequency (100 Hz) produced significantly greater force in EDL compared with higher stimulation frequencies. Therefore, in EDL, the effects of caffeine on PO were greater when the lowest, submaximal stimulation frequency was used.

A caffeine treatment-induced elevation in muscle PO supports the finding of James et al. (16), who also used a physiologically relevant 70 μM caffeine concentration to treat maximally stimulated EDL. The 3.3% increase obtained in EDL in
the present study using the same parameters is similar to the 2–3% increase reported by James et al., but markedly lower than the 6.4% power improvement seen in soleus in the present study. These results from the present study suggest that, in mammals, physiological levels of caffeine treatment will directly induce small increases in PO in short-term, high-intensity activity (e.g., 100-m sprint in athletics); however, it seems likely that caffeine will have greater ergogenic benefit during lower intensity sporting activities that are primarily powered by slow-muscle fiber types.

It has long been established that caffeine can alter excitation-contraction coupling (23). The mechanism by which this increase in PO has occurred in the present study can be attributed to the ability of caffeine to alter intramuscular ion handling. The mechanism for this action of caffeine is still unclear; however, it is believed that caffeine operates directly as an adenosine receptor antagonist on A1 receptors on the skeletal muscle membrane and/or binds to RyR receptors of the SR, as shown in vitro with 10 mM caffeine treatment and in RyR−/− mice (4, 7, 10, 27). These processes probably result in a combination of improved opening of the RyR2 channels of the SR, stimulating a greater release of Ca2+ into the intracellular space, an increase in myofibrillar Ca2+ sensitivity, a decrease in the sensitivity of the SR Ca2+ pump, and an increased SR Ca2+ permeability. Consequently, the rate of Ca2+ efflux from the intracellular space back to the SR may be significantly slower, resulting in a greater basal and activated intracellular Ca2+ concentration, hence increased relaxation time (1, 2). The work loop shapes for both EDL and soleus (Fig. 8) show that caffeine treatment caused a direct increase in muscle force during shortening; however, no appreciable change in relaxation time can be seen. As the muscle was only subjected to four work loop cycles, it is unlikely that the proposed increase in basal Ca2+ between stimulations will occur over this short time period. Fryer and Neering (11) reported that the primary effect of caffeine (0.2–20 mM) on Ca2+ transient was an increase in basal and stimulus-evoked release of Ca2+, accompanied by an elevation of the plateau phase, leading to an increase in twitch and tetanus force in rat EDL and soleus. Magkos and Kavouras (23) further suggested that, if Ca2+ is released from SR at a quicker rate, then this will result in quicker initiation of the Ca2+-induced Ca2+ response mechanism. The primary consequence of these effects is improved cross-bridge kinetics, initially allowing faster and greater availability of the actin binding sites due to a quicker movement of troponin-C, thus promoting greater formation of cross bridges and hence higher force production. Maintaining an elevated concentration of intracellular Ca2+ between stimulus intervals will result in a higher net quantity of calcium when the muscle receives further stimuli. Muscle force is dependent on the concentration of free Ca2+; thus a caffeine-induced elevation in this manner will result in greater force production. With an
increased intracellular Ca²⁺ concentration and a decreased sensitivity of the SR Ca²⁺ pump, the time required to regain intracellular resting concentration of Ca²⁺ and replenish the SR stores of Ca²⁺ will be significantly elongated (2).

Generally, in vivo and in vitro studies report the benefit of caffeine as a group mean (2, 5, 17, 26); however, a degree of interindividual variability in response is common, and studies have shown that not all individuals show a performance improvement (5, 8, 16). In the present study, there were also individual muscles that showed no appreciable change in PO in response to caffeine (Figs. 3 and 4). To the authors’ knowledge, caffeine has not been demonstrated to cause a reduction in acute muscle force; therefore, from a human perspective, micromolar concentrations of caffeine in human blood plasma...
Human physiological concentrations of caffeine are very rarely above 70 μM, with common plasma levels being between 20 and 50 μM (10, 13). The present study indicates that there appears to be a threshold level of caffeine concentration, below which there is no response, and above which there is no further effect of increasing concentration within the physiological range. The effect of 6 or 9 mg/kg body mass caffeine treatment on 2,000-m rowing performance was considered by Bruce et al. (6). The low-dose caffeine trial resulted in a significant 1.3% improvement in time to complete the 2,000 m, while the high dosage resulted in a significant 1% improvement. Above the physiological range, Fryer and Neering (11) demonstrated a dose-dependent potentiation of twitch force in EDL (1–5 mmol/l) and soleus (0.2–1 mmol/l) fibers of rat. James et al. (16) reported that fatigued mouse soleus and EDL produced significantly greater peak stress and PO during shortening of cyclical contractions with 10 mM caffeine treatment compared with those treated with 70 μM caffeine and controls. In conjunction with the present findings, these previous studies suggest that there is no dose-dependent effect of caffeine over the human physiological range.

The effects of caffeine on different muscle fiber types. The ergogenic benefit was significantly greater in mouse soleus (4.7%) compared with EDL (2.5%; Fig. 7). This is comparable to previous evidence, using nonphysiological concentrations of caffeine, in which fast-twitch fibers yielded a greater response to caffeine treatment than slow-twitch fibers. Rossi et al. (27) reported a greater response to 2–30 mM caffeine concentrations in mouse soleus compared with EDL. Fryer and Neering (11) further demonstrated that soleus was more sensitive to a lower dose (200 μM) of caffeine compared with EDL. This can be attributed to muscle-specific differences in Ca^{2+} kinetic properties and muscle-specific expression of RyR isoforms between type I and II fiber types (23). Rossi et al. (27) reported that mouse skeletal muscle RyR3 receptors have a greater sensitivity to caffeine than RyR1. It has been established that muscles with the greatest response have a greater quantity of RyR3. A higher quantity of RyR3 is evident in soleus muscle, explaining the elevated response of soleus in the present study (27).

When relating these results in a broader context to human performance, it should be considered that caffeine has a shorter half-life in rodents, and differences in metabolism also occur between rodents and primates (10). Fibers treated with caffeine may, in vivo, be modulated to produce the same power as controls with the activation of fewer muscle fibers. In vivo, the pattern of fiber stimulation along with length change wave-
forms is likely to be manipulated throughout movement to maximize muscle economy and prevent the onset of fatigue (31). However, these differences are unlikely to affect the overall findings of the study.

In conclusion, physiological levels of caffeine (50 and 70 μM) can directly enhance mouse soleus and EDL muscle PO during short-term cyclical activity. Furthermore, caffeine appears to have no dose-dependent effect on skeletal muscle when used over a relatively small concentration range (50–140 μM). The present study shows that caffeine doses lower than the physiological maximum can produce significant improvements in muscle force. Treatment with 35 μM caffeine showed no appreciable change in the PO of either soleus or EDL; therefore, it is assumed that a relatively high concentration of caffeine is needed to evoke physiological benefit directly at the skeletal muscle. From the results of the present study, it appears that the extent of caffeine-induced potentiation of PO is unlikely to differ between muscle stimulated submaximally compared with maximally.

ACKNOWLEDGMENTS

The authors thank Mark Bodycote and Bethan Grist for technical assistance.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.A.T. and R.S.J. conception and design of research; J.A.T. performed experiments; J.A.T. analyzed data; J.A.T., R.S.J., V.M.C., and M.J.D. interpreted results of experiments; J.A.T. prepared figures; J.A.T., R.S.J., and V.M.C. drafted manuscript; J.A.T., R.S.J., and M.J.D. edited and revised manuscript; J.A.T., R.S.J., V.M.C., and M.J.D. approved final version of manuscript.

REFERENCES