Articles in PresS. J Appl Physiol (October 6, 2011). doi:10.1152/japplphysiol.00801.2011

The Effect of Physiological Concentrations of Caffeine on the Power Output of Maximally and Sub Maximally Stimulated Mouse EDL (Fast) and Soleus (Slow) Muscle

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Caffeine Improves Maximal & Submaximal Muscle Performance

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Abstract

The ergogenic effects of caffeine in human exercise have been shown to improve endurance and anaerobic exercise performance. Previous work has demonstrated that 70μM caffeine (physiological maximum) can directly increase mouse extensor digitorum longus (EDL) muscle power output (PO) in sprint like activity by 3%. Our study used the work loop technique on isolated mouse muscles to investigate whether the direct effect of 70μM caffeine on PO differed between: 1) maximally and sub maximally activated muscle; 2) relatively fast (EDL) and relatively slow (soleus) muscles; 3) caffeine concentrations. 70μM caffeine treatment resulted in significant improvements in PO in maximally and sub maximally 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 ($p<0.001$). 140, 70 and 50μM caffeine treatments 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 power output was higher in muscles with a slower fibre type ($P < 0.001$). Treatment with 35μM 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 power output. This caffeine induced increase in force could provide similar benefit across a range of exercise intensities with greater gains likely in activities powered by slower muscle fibre type.

Key Words: Force, Work Loop, Skeletal Muscle, Sprint, Activation Level

Introduction

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 CNS (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 greater than 30 minutes), power and strength activities.

Following digestion, caffeine can be readily absorbed into the blood stream with peak plasma concentration occurring 30-60 minutes after ingestion (21). Its hydrophobic nature allows free passage of caffeine across all biological membranes resulting in distribution throughout all 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 D_2 receptors (10, 28). Lorist and Tops (21) explored behavioural 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 RYR receptors of the 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 Ca^{2+} 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 & Cupido (29) reported that 6 $mg.kg^{-1}$ body mass (approximately 60µM 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 Ca^{2+} 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 (70µM human *in vivo* maximum, 13) using the work loop technique. They found a small but significant, 2-3%, increase in mean net power output in maximally activated isolated mouse EDL (fast muscle), attributed to increased force production during shortening. 70µM caffeine treatments 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 man, 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 fibre types in the direct effect of physiological concentrations of caffeine on power output. A dose dependant effect on direct muscle performance has further been demonstrated with high 0.07-20 millimolar 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 maximise power output in muscle during physical activity.

The present study aims to investigate whether maximal physiological concentrations (70μM) of caffeine directly affect the power output of isolated skeletal muscle during brief bouts of cyclical activity, being the first such study to compare between: 1) maximally and sub maximally activated muscle; 2) relatively fast extensor digitorum longus (EDL) and relatively slow soleus muscles; 3) micromolar concentrations (35-140μM) 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, UK) were bred and kept at Coventry University. 8 -10 week old mice (body mass = 30.2 ± 0.81g, 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 hind limb then pinned out at approximately its resting length at room temperature (19-21^{o}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 was left attached at the proximal and distal ends. Aluminium foil T-clips were wrapped around each tendon leaving the bone at the back of the clip to help minimise 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 Ltd, UK) and at the opposing end to a motor (V201, Ling Dynamic Systems, UK). 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 ± 0.36^{o}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 fibre itself but stimulated the muscle via the surrounding fluid.

Muscle length and stimulus amplitude (12-16V for soleus; 14-18V for EDL) 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 (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 optimised to yield maximal tetanic force (normally 140Hz for soleus; 200Hz for EDL), following this further tetanic responses were measured at 2 sub maximal stimulation frequencies (70 & 40Hz for soleus; 150 & 100Hz for EDL). A 5 minute rest period was imposed between each tetanus in order 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 whilst undergoing cyclical length changes (16, 18, 19). Here the muscle was held at L_0 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_0 followed by a shortening to 5% shorter than L_0 before returning back to L_0 at a cycle frequency of 5Hz. 5Hz cycle frequency was used for soleus preparations as it represents the cycle frequency that has previously been shown to elicit maximal power output in mouse soleus muscle and is attainable in running mice (3, 15). 5Hz was also used for EDL preparations in order to enable a direct comparison with soleus muscles, however maximal power output for EDL is achieved at 10Hz cycle frequency (15). The strain used comes from previous estimation of strains that produce maximal power output at 5Hz 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, Massachusetts, USA) via a D/A board

(KPCI3108, Keithley Instruments, Ohio, 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 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 power output was achieved.

A burst duration of 100 ms was found to elicit maximal power output 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; optimising this duration maximises power output. Usually a burst duration of 65 ms was found to elicit maximal power output 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 40Hz 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 power output values. If the muscle is was too active during lengthening there is greater resistance to elongate the muscle back to resting length and therefore a decreased net power output. 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 prior to the muscle reaching maximal length, therefore with a stimulus duration of 65ms in soleus, stimulation continues until 45 ms prior to the muscle reaching its shortest length.

Prior to commencement of testing, muscle power output was measured at maximal and sub maximal stimulation frequencies in all the preparations used (140, 70 & 40Hz for soleus; 200, 150 & 100Hz for EDL). The second loop of each set of four work loops was used as an indicative measure for each trial as it didn't prove to be different from loop 3. Following this all the length and stimulation parameters were kept constant and a 10 minute rest between each trial was enforced in order to allow maximal recovery time (17).

Muscle preparations were subjected to 4 work loops at 10 minute intervals over a 120 minute duration. The protocol consisted of 3 control measurements in standard Krebs-Henseleit solution,

followed by 6 measurements in Krebs-Henseleit solution containing 70μM caffeine, concluding with a washout period of 4 measurements in standard Krebs-Henseleit solution. In order to test for a possible interaction between caffeine and simulation frequency this procedure was repeated using 140, 70 or 40Hz and 200, 150 or 100Hz 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 35μM.

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 0.0001g. Mean muscle cross-sectional area was calculated from mean fibre length, muscle mass and an assumed muscle density of 1060 kg m^{-3} (25). 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 $W.kg^{-1}$.

Statistical Analysis of the Data

Single factor analysis of variance (ANOVA) were performed in SPSS (Version 16, SPSS inc., IL, USA) in order to investigate the difference in isometric stress and work loop power between EDL and soleus muscles. Further single factor ANOVA's were performed in order 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; work loop power in EDL.

Prior to commencement of testing, muscle stress and power output at 140Hz, 70Hz and 40Hz for soleus and 200Hz, 150Hz and 100Hz for EDL were measured in all the preparations used. Two factor ANOVA's (2x3 ANOVA's) were conducted on this data in order 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 power output as the dependant variable. Tukey post hoc tests were performed for stimulation frequency where any significant differences were found.

Prior to testing the effect of 70µM caffeine over different stimulation frequencies, there was no significant difference in stress and power output between caffeine treatment categories in soleus and EDL (ANOVA p <0.65 in all cases) prior to caffeine treatment. Prior to testing the effects of different caffeine concentrations there was no significant difference in stress and power output between treatment categories in EDL (ANOVA p = 0.723). In soleus the 50µM treatment group produced significantly more stress than 35µM 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 power output between the treatment categories (p=0.695 in both cases). A reduction in stimulation frequency 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 prior to treatment.

Muscle power output will decrease over time due to the gradual development of an anoxic core. In order to avoid deterioration in muscle performance masking the effects of caffeine, a 1^{st} order regression equation was calculated using the control data and washout data in order to identify the linear relationship between muscle power output and time. This regression equation was then used to determine theoretical control muscle power output 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 - 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 in order 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 (100Hz, 150Hz, 200 Hz) and caffeine treatment (70uM, control) on soleus power output were tested in 2-factor (3x2) ANOVA. The same statistical test was conducted in a separate 2-factor (3x2) ANOVA for the EDL muscle. In order to test for a significant effect of caffeine concentration a further 2 factor (2x3) ANOVA was conducted separately for soleus and EDL. Again power output was the dependant variable with caffeine treatment (70µM caffeine or control) and caffeine concentration as the fixed factors. A significant interaction between concentration and treatment was identified in EDL treated with 70µM caffeine at different stimulation frequencies and both soleus and EDL treated with altered caffeine concentrations (two factor (2x3) 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 to control.

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

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 40Hz and to 91.1% and 69.6% of maximum for EDL when stimulated at 150Hz and 100Hz) for both soleus and EDL (Table 1; two factor (2x3) ANOVA main effect $p<0.001$ in both cases). A reduction in stimulation frequency also resulted in a significant decrease in maximum work loop stress (to 64.3% and 42.9% of maximum for soleus when stimulated at 70 and 40Hz and to 87.1% and 59.5% of maximum for EDL when stimulated at 150Hz and 100Hz) in soleus and EDL (Table 1; two factor (2x3) ANOVA main effect $p<0.001$ in both cases).

Effects of Stimulation Frequency and 70µM Caffeine Treatment on Muscle Power Output

70µM caffeine treatment of soleus elicited significantly greater power output than controls in all cases (Figure 1; single factor ANOVA main effect $p=0.02$). Caffeine elicited a mean peak power output increase of 6.4%, 6.2% and 5.7% for 140Hz, 70Hz and 40Hz stimulation frequencies respectively. There was no significant difference in the effect of caffeine between stimulation frequencies (Figure 1; two factor (2x3) ANOVA main effect $p=0.093$).

70µM caffeine treatment of EDL elicited significantly greater power output than controls (Figure 2; single factor ANOVA main effect $p<0.005$ in all cases). Caffeine elicited a mean increase in peak power output of 3.3%, 4.2%, and 6.7% for 200Hz, 150Hz and 100Hz stimulation frequencies respectively. There was no significant difference in the effect of 70µM caffeine between 200Hz and 150Hz (Figure 2; Tukey $p=0.976$). However, 70µM caffeine treatment elicited a significantly greater increase in PO at 100Hz compared to 200Hz and 150Hz (Figure 2; two factor (2x3) ANOVA Tukey $p<0.005$ in both cases).

A set of responders and a set of non responders (those showing no noticeable improvement in response to treatment) were evident in soleus (Figure 3) and EDL (Figure 4) in all the treatment

groups besides 100Hz sub maximally stimulated EDL. Caffeine treated EDL, stimulated at 100Hz, showed no obvious non responders to the treatment (Figure 4C).

The Effect of 35, 50, 70 & 140μM Caffeine Treatment on Muscle Power Output

Treatment of soleus muscle with 140μM, 70μM and 50μM 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 35μM 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 140μM, 70μM and 50μM caffeine treatments (Fig 5; two factor (2x3) ANOVA Tukey $p<$ 0.473 in all cases).

Treatment of EDL muscle with 140μM, 70μM and 50μM caffeine resulted in a significant increase in mean maximal power of up to 3.3% (Figure 6; single factor ANOVA main effect $p<0.022$ in all cases). Treatment using 35μM caffeine failed to significantly increase EDL muscles maximal PO (Figure 6; single factor ANOVA main effect $p= 0.341$). There was no significant difference in the increase in PO between 140μM, 70μM and 50μM caffeine treatments (Figure 6; two factor (2x3) ANOVA Tukey $p >$ 0.421 in all cases).

As there was no significant difference in response between 140μM, 70μM and 50μM caffeine treatments these results were pooled and soleus was compared against EDL. Treatment of soleus muscles with 50μM - 140μM caffeine resulted in a significantly greater increase in power output (4.7%) compared to EDL (2.5%) muscle (Figure 7; ANOVA two factor (2x3) main effect $p <0.001$)

Discussion

The mean maximal isometric tetanic stress was 189 ± 12 kN m^{-2} and 300 ± 23 kN m^{-2} for soleus and extensor digitorum longus (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 power output was 31.7 ± 1.8 W kg^{-1} and 85.2 ± 7.1 W kg^{-1} (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 power output between studies could be attributed to muscle fibre 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 70μM caffeine on muscle power output at maximal and sub maximal stimulation frequencies.

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

A caffeine treatment induced elevation in muscle power output 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* (16), 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 power

output in short term high intensity activity (e.g.100m 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 fibre types.

It has long been established that caffeine can alter excitation-contraction coupling (23). The mechanism by which this increase in power output 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 10mM 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 Ca^{2+} into the intracellular space, an increase in myofibrillar Ca^{2+} sensitivity, a decrease in the sensitivity of the SR Ca^{2+} pump, and an increased SR Ca^{2+} permeability. Consequently the rate of Ca^{2+} efflux from the intracellular space back to the SR may be significantly slower resulting in a greater basal and activated intracellular Ca^{2+} 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 4 work loop cycles it is unlikely that the proposed increase in basal Ca^{2+} 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 Ca^{2+} transient was an increase in basal and stimulus evoked release of Ca^{2+} accompanied by an elevation of the plateau phase leading to an increase in twitch and tetanus force in rat EDL and soleus. Magkos & Kavouras (23) further suggested that if Ca^{2+} is released from SR at a quicker rate then this will result in quicker initiation of the Ca^{2+} induced Ca^{2+} 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 Ca^{2+} 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 Ca^{2+}, thus a caffeine induced elevation in this manner will result in greater force production. With an increased intracellular Ca^{2+} concentration and a decreased sensitivity of the SR Ca^{2+} pump the time required to regain intracellular resting concentration of Ca^{2+} and replenish the SR stores of Ca^{2+} 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 inter-individual 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 power output in response to caffeine (Fig 3 & 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 can most likely have direct beneficial or negligible effects on skeletal muscle performance.

Tarnpolsky & Cupido (29) suggested that at a sub maximal stimulation frequencies caffeine would promote greater release of Ca^{2+}. The present study doesn't fully support this finding as there were no significant increases in soleus muscle power output with decreased stimulation frequency. EDL showed a similar response when 200Hz (maximal) was compared against 150Hz, however, a significant enhancement in muscle power did occur at 100Hz. For this treatment group there appeared to be no 'non responders' to the caffeine treatment (Fig 4; C) hence the mean increase in power output was significantly higher than at 200Hz and 150Hz. As the precise mechanism of the action of caffeine is still unknown we are unable to suggest that a greater number of responders occur at lower stimulation frequencies. Overall our findings suggest that there is a limit to the level of calcium influx that caffeine promotes and further highlights the need for greater investigation into the mechanisms of the response.

The effects of 35, 50, 70 and 140µM concentrations of caffeine on maximal force production

140µM, 70µM and 50µM caffeine treatment resulted in significant improvements in mean power output of mouse soleus (up to 6%; Fig 5) and EDL muscle (up to 3.3%; Fig 6). There were no significant differences in the level of ergogenic benefit between each concentration. Treatment of soleus and EDL with 35µM caffeine failed to potentiate force.

Human physiological concentrations of caffeine are very rarely above 70µM with common plasma levels being between 20-50µM (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 $mg.kg^{-1}$ body mass caffeine treatment on 2000m 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 2000m whilst the high dosage resulted in a significant 1% improvement. Above the physiological range Fryer and Neering (11) demonstrated a dose dependant potentiation of twitch force in EDL (1-5 mmol l^{-1}) and soleus (0.2 – 1 mmol l^{-1}) fibres of rat. James et al (16) reported that fatigued mouse soleus and EDL produced significantly greater peak stress and power output during shortening of cyclical contractions with 10mM caffeine treatment compared to those treated with 70µM caffeine and controlsIn conjunction with the present findings these previous studies suggest there is no dose dependant effect of caffeine over the human physiological range.

The effects of caffeine on different muscle fibre types

The ergogenic benefit was significantly greater in mouse soleus (4.7%) compared to EDL (2.5%; Fig 7). This is comparable to previous evidence, using non-physiological concentrations of caffeine, where fast twitch fibres yielded a greater response to caffeine treatment than slow twitch fibres. Rossi *et al* (27) reported a greater response to 2-30mM caffeine concentrations in mouse soleus compared to EDL. Fryer and Neering (11) further demonstrated that soleus was more sensitive to a lower dose (200µM) of caffeine compared to 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 fibre 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). Fibres treated with caffeine may *in vivo* be modulated to produce the same power as controls with the activation of fewer muscle fibres. *In vivo* the pattern of fibre stimulation along with length change waveforms are likely to be manipulated throughout movement in order to maximise 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 & 70µM) can directly enhance mouse soleus and EDL muscle power output during short term cyclical activity. Further to this caffeine appears to have no dose dependant effect on skeletal muscle when used over a relatively small concentration range (50-140µM). The current study shows that caffeine doses lower than the physiological maximum can produce significant improvements in muscle force. Treatment with 35µM caffeine showed no appreciable change in the power output 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 current study it appears that the extent of caffeine induced potentiation of power output is unlikely to differ between muscle stimulated sub maximally compared to maximally.

Acknowledgements

The authors would like to thank Mark Bodycote and Bethan Grist for technical assistance. Thanks to two anonymous referees whose constructive comments helped to improve this manuscript.

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Figures

Figure 1. – The mean acute effect of 70µM caffeine on work loop power output in mouse soleus muscle at 140Hz, 70Hz, and 40Hz stimulation frequencies [Data represented as mean & SE] n=8 in each case.

Figure 2. – The mean acute effect of 70µM caffeine on work loop power output in mouse EDL muscle at 200Hz, 150Hz, and 100Hz stimulation frequencies [Data represented as mean & SE] n=8 in each case.

Figure 3. – The acute effect of 70µM caffeine on work loop power output identifying the differences in response to caffeine between individual mouse soleus muscles at 140Hz(A), 70Hz(B), and 40Hz(C) stimulation frequencies. The magnitude of response to caffeine varies between individuals, with some individuals (non-responders) showing no change in power output.

Figure 4. – The acute effect of 70µM caffeine on work loop power output identifying the differences in response to caffeine between individual mouse EDL muscles at 200Hz(A), 150Hz(B), and 100Hz(C) stimulation frequencies. The magnitude of response to caffeine varies between individuals, with some individuals (non-responders) showing no change in power output.

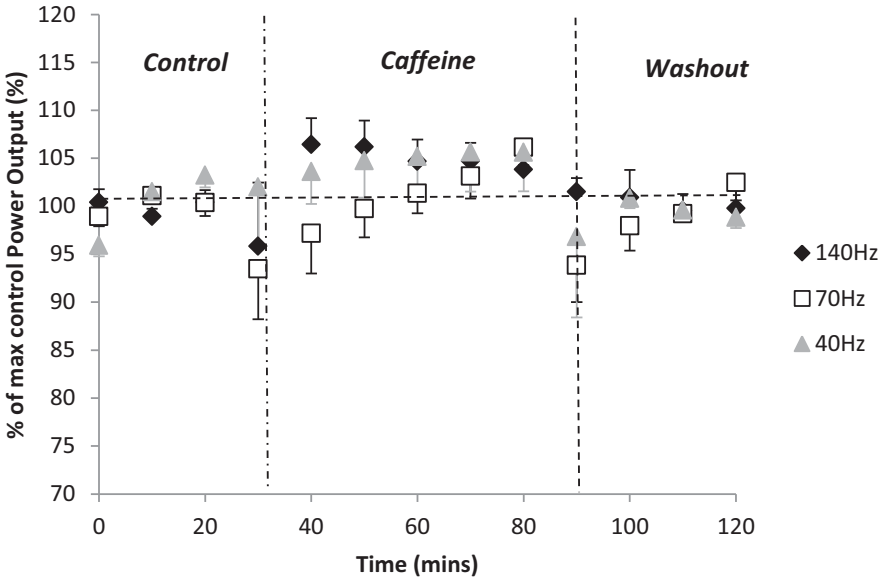
Figure 5. - The mean acute effect of 140, 70, 50 & 35µM caffeine treatment on work loop power output of mouse soleus muscle maximally stimulated at 140Hz stimulation frequency [Data represented as mean & SE] n=10 for 35, 50 and 140µM n=8 for 70µM.

Figure 6. - The mean acute effect of 140, 70, 50 & 35µM caffeine treatment on work loop power output of mouse EDL muscle maximally stimulated at 140Hz stimulation frequency. [Data represented as mean & SE] n=10 for 35, 50, 140µM; n=8 for 70µM.

Figure 7. - The mean effect of caffeine treatment on acute maximal power output of mouse EDL and soleus muscle [140, 70 & 50µM data pooled for each muscle; Data represented as mean & SE; n = 28 in each case]

Figure 8. – Typical effects of caffeine treatment on work loop shapes in mouse EDL (A) and soleus (B) stimulated maximally at 5Hz cycle frequency

Table 1. – The mean effect of altered stimulation frequency on tetanus stress and work loop power in mouse EDL and Soleus.



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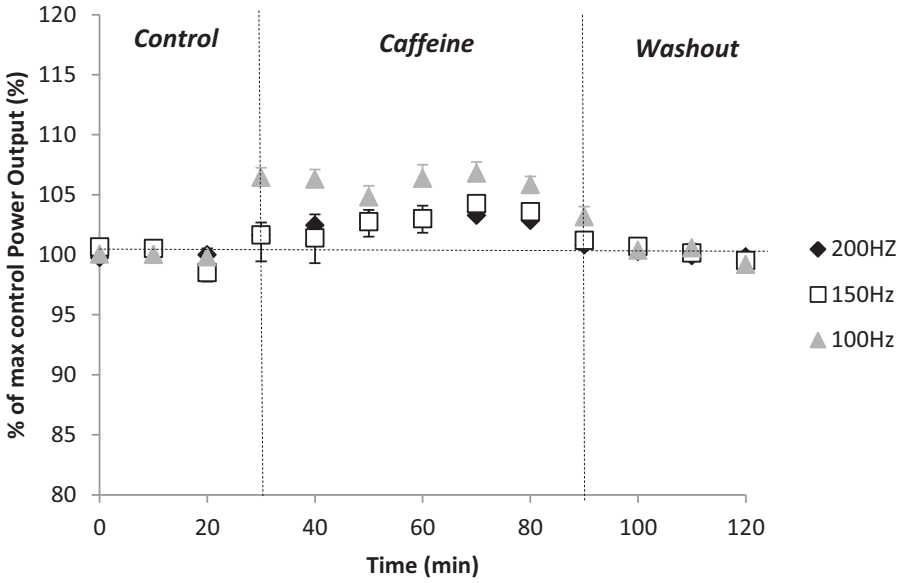
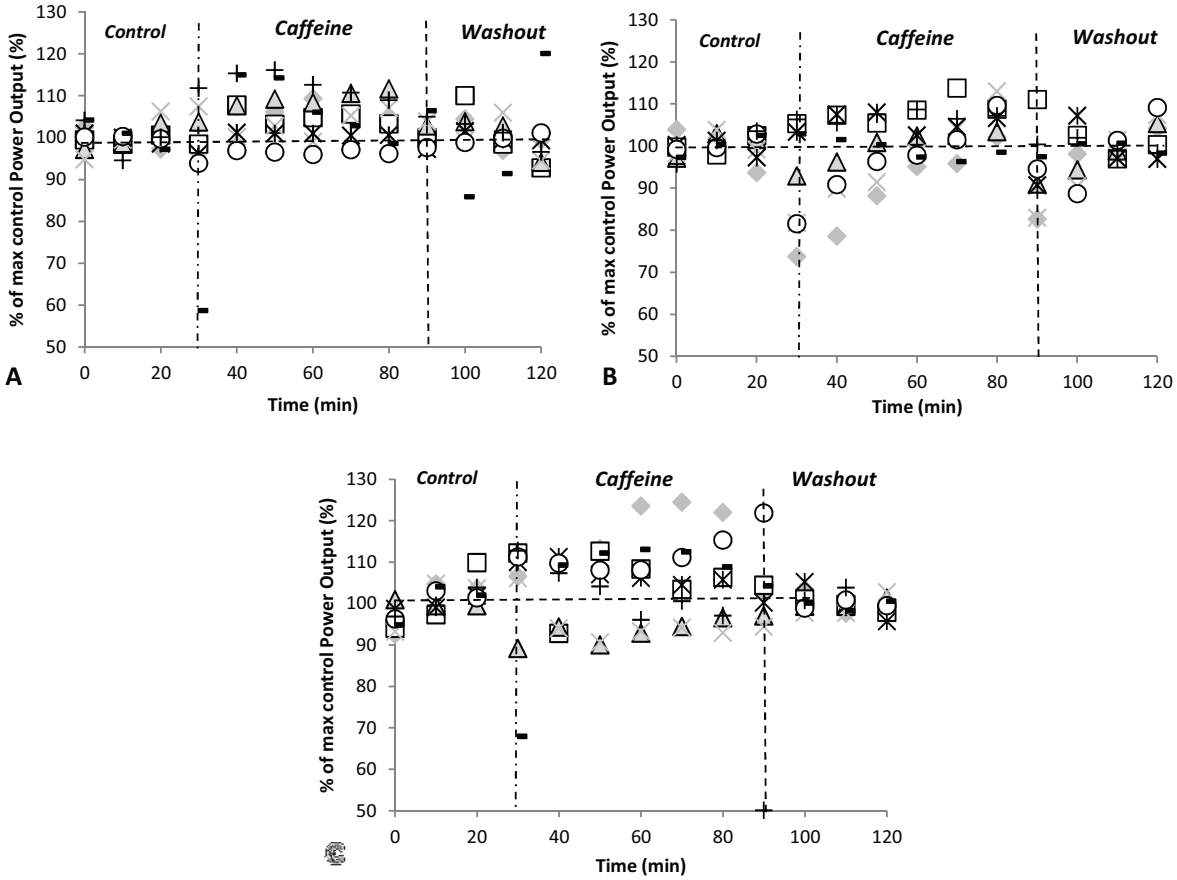


Figure 2. – The mean acute effect of 70µM caffeine on work loop power output in mouse EDL muscle at 200Hz, 150Hz, and 100Hz stimulation frequencies [Data represented as mean & SE] n=8 in each case



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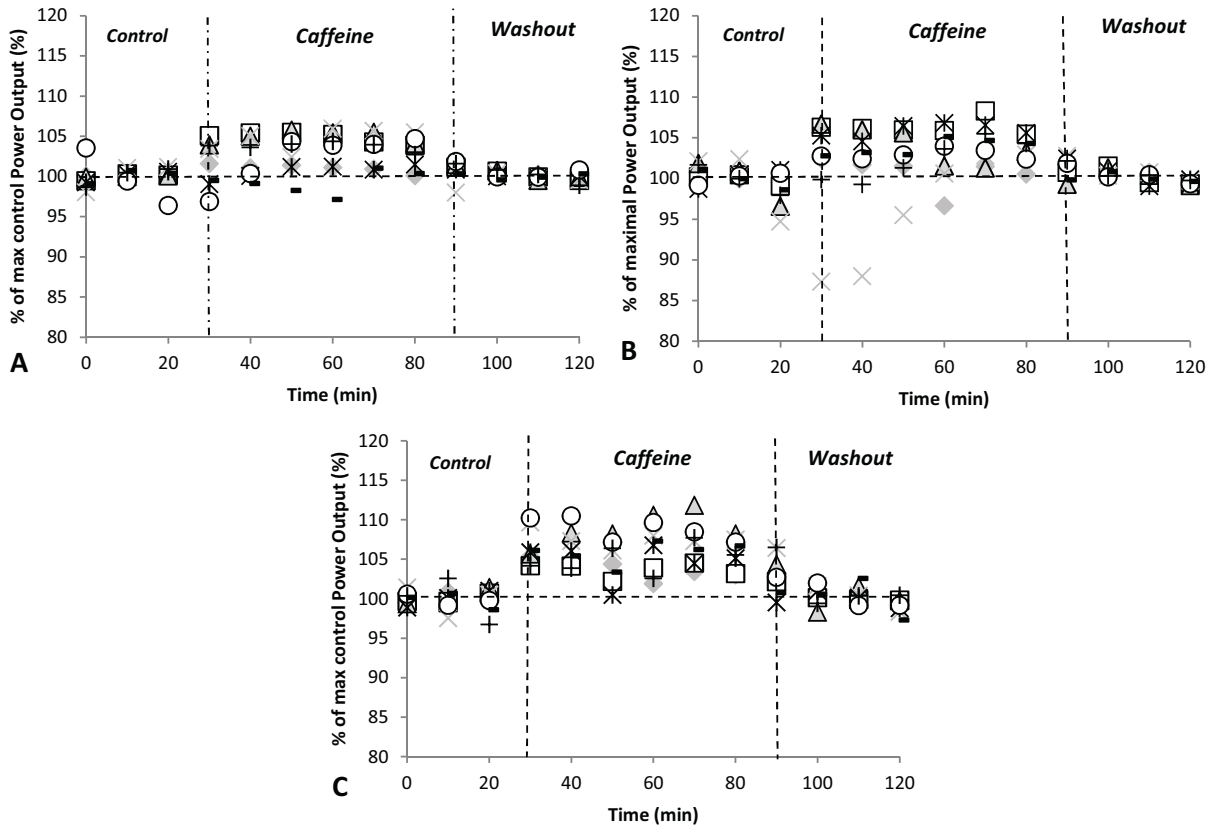

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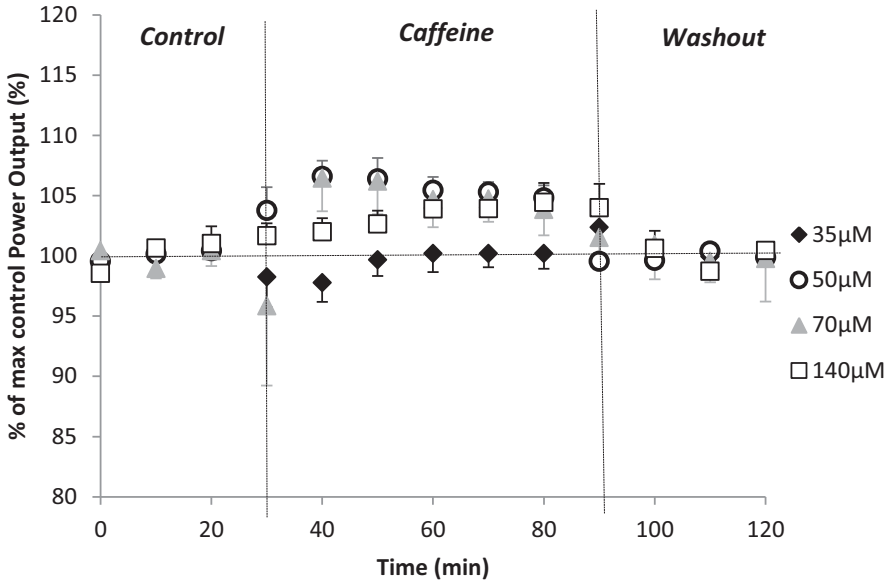

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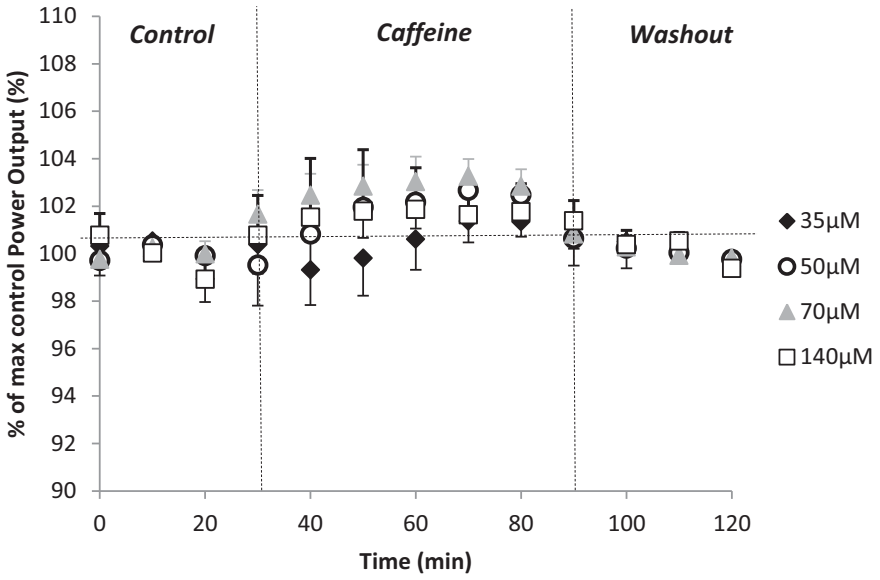

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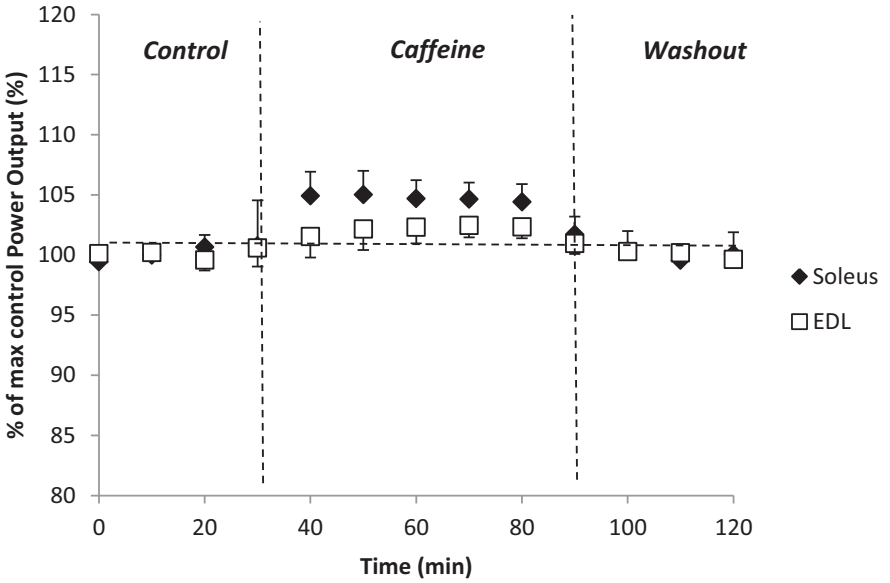

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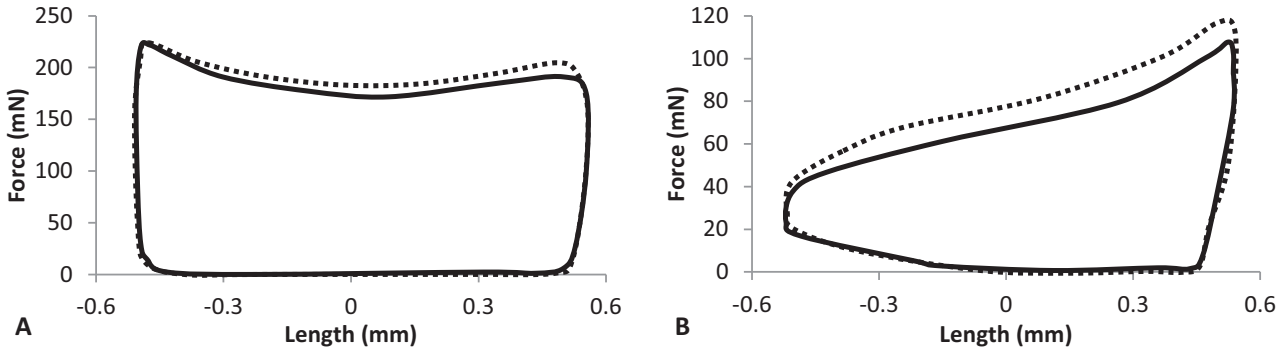

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	Soleus		
Twitch Stress (kN.m^{-2})		32.7±2.6	
Stimulation frequency	40Hz	70Hz	140Hz
Tetanus Stress (kN.m^{-2})	125.8±11	166.2±11.5	189.4±11.9
Max Work Loop PO (W/kg)	13.6±1.2	20.4±1.9	31.7±1.8
	EDL		
Twitch Stress (kN.m^{-2})		66.2±6.2	
Stimulation frequency	100Hz	150Hz	200Hz
Tetanus Stress (kN.m^{-2})	209±22.43	273.9±24.3	300.5±23.2
Max Work Loop PO (W/kg)	50.7±5	74.2±6.4	85.2±7.1

[Data represented as Mean ± SE]