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Is the Ergogenicity of Caffeine Affected by Increasing Age? The Direct Effect of a Physiological Concentration of Caffeine on the Power Output of Maximally Stimulated EDL and Diaphragm Muscle Isolated From the Mouse

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Abstract

Introduction: Caffeine is a well-established performance enhancing nutritional supplement in a young healthy population, however far less is known about how its ergogenicity is affected by increasing age. A recent review has highlighted the value of studies examining the direct effect of caffeine on isolated skeletal muscle contractility, but the present work is the first to assess the direct effect of 70μM caffeine (physiological maximum) on the maximal power output of isolated mammalian muscle from an age range representing developmental to early ageing. *Method:* Female CD1 mice were aged to 3, 10, 30 and 50 weeks (n = 20 in each case) and either whole EDL or a section of the diaphragm was isolated and maximal power output determined using the work loop technique. Once contractile performance was maximised, each muscle preparation was treated with 70μM caffeine and its contractile performance was measured for a further 60 minutes. *Results:* In both mouse EDL and diaphragm 70μM caffeine treatment resulted in a significant increase in maximal muscle power output that was greatest at 10 or 30 weeks (up to 5% & 6% improvement respectively). This potentiation of maximal muscle power output was significantly lower at the early ageing time point, 50 weeks (up to 3% & 2% improvement respectively), and in mice in the developmental stage, at 3 weeks of age (up to 1% & 2% improvement respectively). *Conclusion:* Uniquely, the present findings indicate a reduced age specific sensitivity to the performance enhancing effect of caffeine in developmental and aged mice which is likely to be attributed to age related muscle growth and degradation, respectively. Importantly, the findings indicate that caffeine may still provide a substantial ergogenic aid in older populations which could prove important for improving functional capacity in tasks of daily living.

Key Words: Dynapenia, Ergogenic Aid, Force, Sarcopenia, Skeletal Muscle, Work Loop

Introduction

The ergogenic benefit of caffeine to promote performance enhancing effects has been extensively studied and it is well recognised that caffeine induces increases in endurance, strength and power performance in young healthy participants [see reviews 6, 16, 23]. Given that large amounts of caffeine are consumed by a broad age range of people in western societies, there is surprisingly little evidence examining how the ergogenicity of this drug is affected by age. However, with an increasingly aged population whose decline in skeletal muscle function affects performance in everyday tasks [60], research is warranted to examine the age specific sensitivity of skeletal muscle to the direct performance enhancing effects of caffeine.

Norager et al. [41] was one of the first studies to examine the effect of acute caffeine supplementation on exercise performance in an elderly population. The study reported a significant increase in cycling endurance (25%), arm flexion endurance (54%) and associated reduction in RPE in men and women aged over 70 following 6mg/kg caffeine consumption. No significant differences were found in muscle strength, walking speed, or reaction time. Interestingly, Norager et al, [41] reported that the increase in arm flexion endurance was markedly higher than that seen in an earlier study on younger individuals. This suggests that caffeine may prove to be more ergogenic in an older adult population. In support of this notion, Swift and Tiplady [52] further concluded that the elderly may be more sensitive to the effects of caffeine at the psychomotor level with this population showing greater improvements in attention and choice reaction time compared to younger participants. Duncan et al, [20] demonstrated that low dose (3mg/kg) caffeine improved six minute walk distance, 8 foot up and go time, number of arm curl reps completed in 30 seconds and manual dexterity in participants aged 66 years. Subsequently Duncan et al, [20] concluded that caffeine could be used as an effective nutritional supplement for improving functional performance in the elderly. In agreement, Momsen et al, [39] demonstrated a significant increase in maximal walking distance (20%) and maximal isometric knee extension strength (9.8%) following 6 mg/kg caffeine supplementation in 68 year old patients with moderate intermittent claudication. Conversely, evidence suggests that a caffeine induced increase in muscular strength of elderly participants is not always reported [54]. This

relatively small quantity of research and the ambiguity in findings warrant further more controlled research examining the relationship between the performance enhancing effects of caffeine and increasing age.

A recent review by Tallis et al, [53] has highlighted the value of isolated mammalian muscle studies for examining the direct performance enhancing effect of caffeine. As the mechanism of enhanced performance has in part been attributed to the direct action of caffeine to promote an increase in muscle function [37], such in vitro studies allow the direct assessment of caffeine on skeletal muscle without interference from other central and physiological processes. More importantly digestion and distribution, habituation, withdrawal effects, side effects of high dose caffeine consumption and motivation to complete repeated maximal exercise, that are factors difficult to control in human studies, are not prevalent in isolated muscle work. Such aspects have been suggested to result in an individual caffeine response subsequently resulting in a number of equivocal findings in the human literature [53]. The same limitations affect the relatively small amount of literature examining the effect of caffeine on performance in older human participants and subsequently warrant the use of isolated muscle studies to get a clearer indication of the effectiveness of this drug in such populations.

Previous work, using isolated muscles from young healthy rodents, has demonstrated that high (mM), and more recently, physiologically relevant (50-70μM) concentrations of caffeine can cause a significant fibre type specific improvement in the maximal force and power producing properties of skeletal muscle [3, 28, 29, 55, 56]. Mechanistically this has been attributed to the action of caffeine as an adenosine receptor antagonist acting specifically at the A1 receptors on the skeletal muscle membrane and/or its ability to bind to ryanodine receptors (RYR) of the sarcoplasmic reticulum (SR) [14, 21, 46]. The net effect is an improvement in excitation contraction coupling promoting greater SR Ca^{2+} release, and as a consequence, a more forceful contraction [23, 33, 55]. An increase in age is associated with a reduction in the efficacy of the excitation contraction coupling process and altered Ca^{2+} handling properties as a mechanism for the age related loss of muscle function [17, 40]. These age related changes in E-C coupling may significantly reduce the skeletal muscle response to caffeine

106 treatment and mechanistically would appear to contradict the increased caffeine sensitivity proposed
107 by Norager et al, [41].

108 The present study builds on our previous body of work (reviewed in [53]) and is the first to assess the
109 direct effects of a physiologically relevant concentration of caffeine (70μM, maximal for human
110 consumption; [21]) on mammalian skeletal muscle performance in a large age range of individuals.
111 Specifically our aim was to measure the maximal power output of isolated mouse EDL
112 (predominantly fast-twitch) and diaphragm (mixed muscle fibre type) skeletal muscles over an age
113 range from 'developmental' (3 weeks old) to 'early aged' (50 weeks old). Previous evidence has
114 demonstrated that 50 week old CD1 mice show a substantial age related decline in skeletal muscle
115 mechanical performance [57] and were subsequently deemed appropriate for use in the context of this
116 study. Moreover, the present work is the first (*in vivo or in vitro*) study to examine the ergogenic
117 effect of caffeine over a broad range of ages, rather than just examining older adults or younger adults
118 as in previous human work [6, 20, 23, 41, 56]. By using the work loop technique to examine different
119 muscles, the present study will be able to establish fibre type specific effects of caffeine on the
120 mechanical properties of muscle in relation to age using a method that better simulates real life
121 muscle function than more commonly used in vitro tests [27, 30, 31, 32]. In addition, the ergogenic
122 effect of caffeine on exercise performance has not been studied in children and adolescents despite the
123 high consumption of caffeinated products in this population [5]. The present study will offer an
124 important insight into the direct effects of caffeine in juvenile and elderly populations.

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Methods

Animals

Female white mice (strain CD1 mice, Charles River, UK) were bred and kept in house at Coventry University. This study was approved by the Coventry University ethics committee. The assessment of the age related direct effect of caffeine was conducted on mice aged 3, 10, 30 and 50 weeks old (n=20 in each case). From birth animals were kept in groups of 8 without access to running wheels. The rationale for the use of animals at these age groups to examine the ageing effect on mechanical performance of skeletal muscle is given in our previous work [57] but can be summarised as follows for both EDL and diaphragm: At 3 weeks of age mice are weaned and muscle performance is low, at 10 weeks muscle performance peaks, at 30 weeks the onset of ageing begins and by 50 weeks there is a substantial age related reduction in the maximal force and power generating capacity of various skeletal muscles.

Dissection

Mice were weighed to the nearest 0.1g on an electronic balance (body mass (g): 3 weeks = 15.8 ± 1.2; 10 weeks = 31.9 ± 0.8; 30 weeks = 41.6 ± 0.8; 50 weeks 58.2 ± 5.4; mean ± SE, n=20 in each age group). Mice were then killed by cervical dislocation in accordance with the British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1. Either a ventral section of the costal diaphragm or an EDL muscle was dissected from each mouse in cooled (4-6°C) 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. As a method to prevent tendon slippage during muscle force production, aluminium foil T-clips were wrapped around each tendon of the EDL. The central section of the left hand half of the diaphragm muscle was prepared in a similar fashion, however at one end the two ribs that anchored the muscle were left intact.

Experimental Set-Up

Using either the aluminium foil T-clips or bone, each muscle preparation was connected via crocodile clips to a force transducer at one end (UF1, Pioden Controls Ltd, Kent, UK) and a motor at the opposing end (V201, Ling Dynamic Systems, Hertfordshire, UK). Position of the motor arm was detected via a Linear Variable Displacement Transformer (DFG5.0, Solartron Metrology, Sussex, UK). The muscle was maintained in circulated oxygenated Krebs-Henseleit solution at a constant temperature of 37^{o}C. The muscle was electrically stimulated to produce force via parallel platinum electrodes inside the muscle chamber. These electrodes were not directly in contact with the muscle or the nerve branch but caused activation by stimulating the surrounding fluid. Muscle stimulation and length change parameters were controlled using custom written software (Testpoint, CEC, Massachusetts, USA) via a D/A board (KPCI3108, Keithley Instruments, Ohio, USA) on a desktop PC. Each muscle preparation went through a series of isometric muscle tests followed by assessments of work loop power.

Isometric Testing

Initially twitch force was assessed by electrically stimulating the muscle whilst held at a constant length. The muscle length and stimulation amplitude (14-18V for EDL; 10-16V for diaphragm) were optimised to produce maximal twitch force. The muscle length that corresponded with maximal twitch force was measured using an eyepiece graticule and defined as L_0. Similarly to James *et al* [27], mean muscle fibre length was calculated as 75% of L_0 for EDL muscle. As no such estimate of fibre length exists for diaphragm the direct measurement taken was used as L_0 [57]. Each muscle was then subjected to a 250ms burst of electrical stimulation in order to produce a tetanus response. Stimulation frequency was optimised in order to produce maximal tetanus force (usually 200Hz for EDL and 140Hz for diaphragm; this was not affected by age). A 5 minute rest period was imposed between each tetanus in order to allow the muscle sufficient recovery between stimulations.

The Work Loop Technique

The work loop technique was employed as a method of providing a closer representation of *in vivo* skeletal muscle performance [31]. Similarly to *in vivo* muscle function, this method considers that

muscle cannot shorten indefinitely and must go through a period of re-lengthening before subsequent contraction. The work loop technique employs cyclical length changes using waveforms and stimulation parameters that more closely approximate those used *in vivo* to assess the ability of the muscle to produce dynamic power [28, 30, 31, 32].

Essentially the approach used for EDL and diaphragm was the same as that used in our previous study on ageing [57]. The muscle was held at the previously determined L_0 and the stimulation amplitude and stimulation frequency parameters that yielded maximal tetanic force were employed. Each muscle was subjected to four sinusoidal length change cycles per set at a total symmetrical strain of 0.10, as this strain has been used previously to elicit maximal power output in these muscles [27, 55, 57]. As such, the muscle lengthened by 5% from L_0 followed by a shortening to 5% shorter than L_0 before returning back to L_0. A cycle frequency of 10Hz and 7Hz was used for EDL and diaphragm muscle respectively. 10Hz represents the cycle frequency that has previously been shown to elicit maximal power output in EDL [27]. 7Hz represents the cycle frequency that elicited maximal power output in diaphragm in preliminary work by the authors and is similar to previous findings [4]. The magnitude and frequency of length changes and electrical stimulation were controlled via the Testpoint software. 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 [31]. Stimulus burst duration (stimulation primarily through shortening) was altered until maximal net power output was achieved.

Usually a 49 ms burst duration was used for EDL which is in keeping with that previously used at 10Hz cycle frequency [27, 57]. The burst duration commonly used to elicit maximum power output in diaphragm muscle was 55 ms, similar to our previous work [57]. On occasions the burst duration had to be increased or decreased to adjust the number of stimuli given. This was determined by examining the work loop power output and by interpretation of the work loop shapes. e.g. if the muscle is too active during re-lengthening it will significantly distort the shape of the loop and reduce muscle power output by increasing the resistance of the muscle to stretch. A stimulation phase shift of -2 ms and -5 ms were used for EDL and diaphragm respectively, as they corresponded with maximal power output,

209 in agreement with our previous work [57]. So for EDL this stimulation phase shift value dictates that
210 stimulation of the muscle starts 2 ms prior to the muscle reaching maximal length.

211 *The Effect of 70μM Caffeine Treatment*

212 Each muscle was subjected to a set of 4 work loop cycles every ten minutes over a 130 minute
213 duration [28, 55, 57]. Three measurements of the muscles maximal power output were made in
214 standard Krebs-Henseleit solution and this formed the initial control baseline measurement. Following
215 this the circulating fluid was changed to Krebs containing 70μM caffeine and a further 6
216 measurements of maximal power output were made. The assessment concluded with a washout period
217 where the circulating fluid was replaced with standard Krebs and a further 4 measurements of
218 maximal power were taken, the latter three of which were used to represent the washout control.

219 *Muscle Mass Measurements and Dimension Calculations*

220 At the end of the experiment the muscle was removed from the clips and the tendons were removed
221 leaving the whole muscle intact. The muscle was then blotted on absorbent paper to remove excess
222 fluid. The muscle was then placed on an electronic balance (Mettler Toledo B204-S, Zurich,
223 Switzerland) to determine the wet muscle mass to the nearest 0.0001g. Mean muscle cross-sectional
224 area was calculated from mean fibre length (muscle length in the case of diaphragm), muscle mass
225 and an assumed muscle density of 1060 kg m^{-3} [38]. Isometric stress was calculated as maximal
226 tetanic force divided by mean muscle cross-sectional area. Muscle power output was normalised to
227 muscle mass to express power as $W.kg^{-1}$.

228 *Statistical Analysis of the Data*

229 In control conditions muscle power output will decrease over time due to the gradual development of
230 an anoxic core [8]. Over the 130 minute duration of the protocol used in the present study, muscle
231 power output had decreased to 92.5±0.61% of initial power output. In order to avoid the given
232 deterioration in muscle power output masking the effects of the caffeine treatment, a 1^{st} order
233 regression equation was calculated using the pre-treatment control data and post treatment washout

control data 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 [28, 55].

Initially for each treatment group, pre-treatment controls were compared directly against post treatment washout controls using a paired t-test. There was no significant difference between these measurements (Paired T-test $p>0.7$ in each individual case) therefore these results were pooled to form controls. Thereafter, it was assumed that any subsequent change in muscle power output was solely the effect of the given caffeine treatment. Consequently controls were compared directly against caffeine treatment using a further paired T-test for each treatment group. To establish whether a difference in the effect of caffeine occurred between ages a single factor ANOVA was conducted on the caffeine treatment data, after normalisation as a percentage of the theoretical control, for each of EDL and diaphragm muscle respectively. When the ANOVA indicated a significant difference between age groups, Tukey post hoc tests were used to identify where these differences occurred. An independent samples t-test was conducted to compare the caffeine treatment results for EDL and diaphragm of the same age group in order to identify whether the ergogenic benefit was greater in a particular muscle at each age.

As the caffeine response was not uniform within each treatment group and EDL muscle mass was significantly affected by age (ANOVA $p<0.001$), we considered it valuable to determine if the caffeine effect was affected by muscle mass. This relationship was determined by using Pearson's two tailed correlation analysis for these data for each treatment group.

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

Results

The Effect of 70μM Caffeine Treatment on the Maximal Power Output of Mouse EDL

Treatment of mouse EDL with 70μM caffeine resulted in a significant increase in power output by up to 1%, 4%, 5% and 3% for 3, 10, 30 and 50 week old mice respectively (Fig 1; paired t-test $p<0.001$ between control and caffeine power output in all cases). There was a significant effect of age on the caffeine induced improvement in power output (ANOVA $p<0.001$). The caffeine induced increase in muscle PO was the highest at 30 weeks and was significantly greater than the response at 3 and 50 weeks (Fig 1; Tukey $p<0.001$ in both cases) and had a tendency to be greater than that at 10 weeks (Fig 1; Tukey $p=0.079$). The ergogenic benefit at 3 weeks was significantly lower than at all other ages (Fig 1; Tukey $p<0.005$ in all cases). The increase in muscle PO at 10 weeks did not prove to be significantly different to that at 50 weeks (Fig 1; Tukey $p=0.733$).

The Effect of 70μM Caffeine Treatment on the Maximal Power Output of Mouse Diaphragm

Treatment of mouse diaphragm with 70μM caffeine resulted in a significant increase in power output by up to 2%, 6%, 4%, and 2% for 3, 10, 30 and 50 week old mice respectively (Fig 2; paired t-test $p<0.001$ in all cases). There was a significant effect of age on the caffeine induced improvement in power output (ANOVA $p<0.005$). The caffeine induced increase in power output at 10 weeks was the highest and was significantly greater than at 3, 30 and 50 weeks (Fig 2; Tukey $p<0.004$ in all cases). Power output was also significantly higher at 30 weeks compared to 3 weeks and 50 weeks (Fig 2; Tukey $p<0.02$ in both cases). There was no significant difference in power output between 3 weeks and 50 weeks (Fig 2; Tukey $p=0.864$).

The ergogenic benefit of 70μM caffeine treatment was significantly greater in EDL muscle compared to diaphragm at 30 and 50 weeks (Fig 3; two-sample t-test $p<0.005$ in both cases). The caffeine induced potentiation of diaphragm power output had a tendency to be greater than EDL at 10 weeks (two-sample t-test $p=0.054$). The caffeine induced increase in maximal muscle power output at 3 weeks was not significantly different between muscles (independent samples t-test $p=0.54$).

The Effect of 70μM Caffeine Treatment in Relation to Muscle Mass

EDL muscle mass was significantly affected by age (ANOVA $p<0.001$). Muscle mass at 3 weeks (0.00672 ± 0.00171g), was significantly lower than that at 10 (0.01244 ± 0.00038g), 30 (0.01422 ± 0.00023g), and 50 (0.00245 ± 0.00078g) weeks (Tukey $p<0.001$ in all cases). Muscle mass at 30 and 50 weeks was significantly greater than that at 10 weeks (Tukey $p<0.001$). Similar comparisons for diaphragm cannot be made due to usage of only part of the diaphragm. There was no significant relationship between whole EDL muscle mass, or the mass of the diaphragm strip, used in the assessment of muscle mechanics and the performance enhancing benefit of caffeine for any of the experimental groups (Table 1; $p>0.07$ in all cases). Therefore, as all of our power output values are expressed relative to control muscle power output the variation in muscle mass between preparations should not need to be further accounted for in our analysis.

Discussion

The Direct Effect of 70μM Caffeine on Aged Skeletal Muscle

The present findings are the first to demonstrate that 70μM caffeine can directly potentiate skeletal muscle power output across a broad range of ages and subsequently adds further evidence to the small body of human research that suggests that caffeine could be used as an effective performance enhancing nutritional supplement in older adult populations [20, 39]. The present work is the first to assess how the sensitivity of the caffeine effect on exercise changes with age. The results demonstrate that the magnitude of the performance enhancing effect is influenced by age and muscle fibre type composition. The caffeine induced potentiation of both EDL and diaphragm muscle power output was significantly reduced in the oldest and youngest age groups compared to the younger adult groups.

The caffeine induced increase in EDL muscle power output at 10 weeks of age (up to 4%) is consistent with the 3% reported by James et al, [28] and Tallis et al, [55] using similar experimental methods. However, caffeine treatment caused the greatest increase in muscle power output at 30 weeks of age. This is particularly interesting given that the onset of the early age related decline in contractile performance has been demonstrate to occur at 30 weeks of age in this strain of mice (see [57] for a full report of the effect of increasing age on mechanical performance of skeletal muscle used in this study). The present work is the first to assess the direct effect of 70μM caffeine on diaphragm muscle and the 6% increase in muscle power is comparable to that previously reported for mouse soleus [55]. In mice both soleus and diaphragm have previously been shown to be composed of more aerobic fibres than EDL [1, 7]. Unlike in EDL, an increase in age beyond that associated with peak mechanical performance of diaphragm [57] caused a significant reduction in the magnitude of the caffeine effect.

This age related reduction in skeletal muscle caffeine sensitivity occurs independently of changes in muscle mass (Table 1). One may speculate that the same absolute dose of caffeine may provide less of a benefit to larger muscles (as in the older age groups) due to a smaller concentration per area of tissue, but this did not prove to be the case. Therefore, it seems likely that skeletal muscle ageing may

be accountable for this observed loss in the ergogenic effect in the oldest mice used in the present study.

The rate of muscle activation and force production is primarily determined by effectiveness of excitation contraction coupling and the intramuscular Ca^{2+} transient time [9]. The demonstrated caffeine induced increase in maximal power output is likely to occur due to the effects of caffeine as a modifier of excitation-contraction coupling [23, 33]. Mechanistically, it is believed that caffeine acts as a direct agonist to adenosine receptors on the skeletal muscle membrane and has been demonstrated to bind to ryanodine receptors (RyR) of the sarcoplasmic reticulum (SR) [10, 14, 15, 21, 46]. It is likely that these actions result in an improved opening of RyR channels promoting a greater Ca^{2+} release into the intracellular space, an increased Ca^{2+} myofibrillar sensitivity, a decreased SR Ca^{2+} pump sensitivity and increased SR Ca^{2+} permeability. Consequently a reduction in the rate of Ca^{2+} efflux from the intracellular space back to the SR is likely to occur resulting in an elevated basal and subsequently activated intracellular Ca^{2+} concentration [2, 3]. Mechanical data from our previous work examining the effect of 70μM caffeine on the maximal power output of isolated skeletal muscle supports this, demonstrating an increase in work during the shortening phase of the work loop [55], and an increase in relaxation time and subsequent work required to re-lengthen the muscle at the end of the shortening phase during fatigue testing [56].

The age related reduction in the contractile performance of the muscles in the present study is believed to result from a reduction in the function of excitation-contraction coupling, as no substantial changes in either EDL or diaphragm fibre type composition or metabolic capacity were found between these same age groups in a previous study [57]. Renganathan et al, [45] demonstrated a significant age related reduction in SR Ca^{2+} release of rat EDL and soleus muscle attributed to DHPR-RyR uncoupling. This was further supported by Delbono et al, [17] who confirmed a similar effect in fast fibres of human quadriceps muscle. A reduction in the voltage gated SR Ca^{2+} release mechanism would result in a decreased Ca^{2+} availability for the contractile proteins and consequently a reduction in contractile force. Therefore, in the context of the present study, caffeine may still bind to RyR in the aged muscle but the age related uncoupling of DHPR-RyR may result in a decrease in the force

and power enhancing effects of caffeine. In addition Larsson & Salviati [35] reported an age related reduction in SR Ca^{2+} concentration of fast twitch muscle of chemically skinned rats. As such it is likely that the reduction in the ergogenic properties of caffeine in aged muscle arise from a reduced ability to increase SR Ca^{2+} release.

The Direct Effect of 70μM Caffeine on Developing Skeletal Muscle

Despite the high consumption of caffeine in children, and caffeine containing products marketed specifically at this age group, the effect of caffeine on exercise performance in this population is not well understood [5]. Turley, Bland and Evans [58] reported limited effects of low, mild and high dose caffeine (1, 3 and 5 $mg.kg^{-1}$) on physiological responses to exercise in 7-9 year old children. Caffeine treatment had no effect on substrate metabolism, however, mild and moderate dose caffeine treatment resulted in a slight increase in blood pressure and a slight decrease in heart rate.

The present study shows that the treatment of muscle from 3 week old mice with 70μM caffeine has only a very small, but significant, effect on the potentiation of maximal work loop power output. The ergogenic benefit in this age group was not significantly different between diaphragm and EDL. Schiaffino and Margreth [48] and Luff and Atwood [36] established that at birth skeletal muscle SR is sparse and occurs as a loose network of tubes. Furthermore Luff and Atwood [36] demonstrated a muscle fibre type specific increase in mouse SR from 1.1% of fibre volume at birth to 5.5% in adult EDL (predominantly fast-twitch), and from 1.7% at birth to 2.9% in adult in soleus (predominantly slow-twitch). SR development occurred most rapidly in the initial 20 days and had reached adult values by 30 days in soleus and was still increasing after 60 days in EDL. Therefore it should be considered that in the 3-week-old diaphragm and EDL muscle used in present study, SR is not fully developed, thereby possibly limiting the action of caffeine on RYR and consequently reducing the level of caffeine induced Ca^{2+} release and subsequent force potentiating effects.

In line with previous literature the present work infers that caffeine has limited effect on physiological responses to exercise and skeletal muscle performance in a pre-mature age group. However the stimulant effects at the central nervous system should be fully evaluated *in vivo* before conclusions are

made about the value of caffeine as an ergogenic aid in children. Hughes and Hale [26] reported only slight improvements in vigilance performance and decreased reaction time in this population; however the effect of caffeine on the cognitive function of children requires further investigation.

The Muscle Specific Action of 70μM Caffeine Treatment

At 10 weeks of age the caffeine induced potentiation of maximal skeletal muscle power output was significantly greater in diaphragm muscle compared to EDL. However, beyond this the effect of caffeine was greater in aged EDL. It has been firmly established in adult rodent models that the force potentiating effects of caffeine are more greatly pronounced in muscle with a predominantly slower fibre type [22, 46, 55]. Although of mixed muscle phenotype, diaphragm will consist of a significantly greater proportion of oxidative muscle fibres than EDL which subsequently may explain why the caffeine induced benefit was greater in this muscle at 10 weeks of age (Figure 3). One may therefore argue that the widely reported age associated increase in type I fibre expression [18] would actually increase caffeine sensitivity. The likely absence of an age related change in fibre type expression in the muscles used in the present study, and the reported significantly greater loss in mechanical function in the diaphragm [57], likely explain why caffeine sensitivity was more dramatically reduced in this muscle.

Irrespective of age the present study is the first to examine the direct effect of a physiologically relevant dose of caffeine on the mechanical performance of the diaphragm. Kivity et al, [34] demonstrated that high dose caffeine significantly improved the pre and post exercise forced expiratory volume in one second (FEV1) in healthy human male subjects, which was largely attributed to caffeine induced bronchodilation. In alignment with the work of Brinbaum and Herbst [11], these findings suggest that caffeine may evoke a significant increase in respiratory function across a range of ages which will have profound effects on exercise capacity by working to meet the oxygen demand of the active tissue. Our findings, combined with those of the previous studies suggest that caffeine may be effective in reducing the functional strain on patients with age associated respiratory diseases such as COPD.

Practical Implications and Limitations of the Present Findings

The present findings have used a more controlled approach to demonstrate that caffeine could be used as an effective nutritional supplement to directly improve muscle function across a broad range of ages. Although the magnitude of the effect was reduced in the older age groups, this small but significant benefit could still be effective in evoking meaningful improvements in functional performance. When the *in vivo* applications are considered, such increases in muscle power output are likely to transfer to an improved resistance to fatigue as caffeine treated muscles will be able to produce the same relative power output with a smaller number of recruited fibres.

In line with our previous work the present results demonstrates an individual response to the caffeine treatment ranging between a 0-11% increase in power in the young adult group [55], however, the present study shows a lower effect, 0-5%, in the older adult group. These findings are particularly interesting given that individual responses have, in human literature, been attributed to caffeine habituation [49]. As this factor is not prevalent in a rodent model it appears that the individual responses to the performance enhancing effect of caffeine needs further investigation. The present results imply that there is no 'one size fits all' approach for maximising the caffeine response as some individuals may have a large increase in performance whilst others demonstrate little or no effect, regardless of previous caffeine exposure.

In addition, caffeine has been shown to promote performance enhancing effects through its action as a central nervous system stimulant across a range of ages [23, 44]. This coupled with the increase in skeletal muscle performance reported in the present study could evoke more substantial increases in functional performance than that demonstrated by our present approach of examining the direct effects on skeletal muscle alone.

Conceptually there is difficulty in relating the 70μM caffeine concentration used in the present work to quantities of oral caffeine consumption due to individual differences in the rate of caffeine metabolism [51], and the need to administer caffeine relative to body mass rather than as an absolute dose. Graham [23] stated that normal plasma caffeine concentrations range between 20-50μM and

Van Soeren and Graham [59] demonstrated that 6 mg/kg caffeine consumption resulted in blood plasma level of ~50μM in athletes aged 37 years. Such moderate concentrations are used regularly in human literature, thus it is conceivable that a blood plasma concentration of 70μM can be achieved using high, but safe oral doses of caffeine. Our previous work using 10 week old mice has shown that caffeine induced improvements in contractility demonstrated at 70μM are still prevalent at 50μM [53]. There is no reason to assume that similar doses will not be equally effective in this ageing model.

Despite findings demonstrating the benefits of caffeine consumption, the older adult population should exercise caution when consuming high quantities of caffeine. In some individuals, high doses of caffeine can lead to increased anxiety, gastrointestinal discomfort, and impairment of fine motor control [12, 50]. Evidence has demonstrated that daily consumption of high doses of caffeine will accelerate bone mineral density (BMD) loss in healthy postmenopausal women with calcium intakes below the recommended daily intake [25], however this has not been associated with an increased risk of bone fracture [24]. Although caffeine may increase blood pressure in non-habitual users, recent evidence demonstrated that heavy coffee consumption is not associated with an increased risk of coronary heart disease and that moderate consumption may actually negate this risk [13, 19]. Evidence further suggests that caffeine may have protective effects on late life cognitive decline, dementia [47, 42] and can act as a therapeutic tool in Parkinson's disease [43].

The present work examines the caffeine response during early ageing, however future work should investigate skeletal muscle responses at the later extremities of older ageing. Although it may be considered that the performance enhancing benefit would decrease if the current trend is continued, an age related shift to a slower muscle fibre type composition could mitigate such a response.

Conclusion

The methods employed in the present study have allowed a more controlled investigation of how the direct performance enhancing effect of caffeine is affected by increasing age. Our results demonstrate that the direct treatment of skeletal muscle with, physiologically relevant, 70μM caffeine significantly

potentiated the maximal power output of mouse EDL and diaphragm muscle independent of age. As such, caffeine may be used as an effective performance enhancing nutritional supplement in an older adult population. This is particularly significant given the equivocal findings reported in the small quantity of whole body human studies examining the effects of caffeine in the elderly. Interestingly however, our results are the first to demonstrate that the ability of caffeine to produce such effects is significantly reduced in this older age group, when compared to younger adults, which is likely to be related to the age related reduction in muscle function caused by a reduction in the efficiency of the excitation contraction coupling process.

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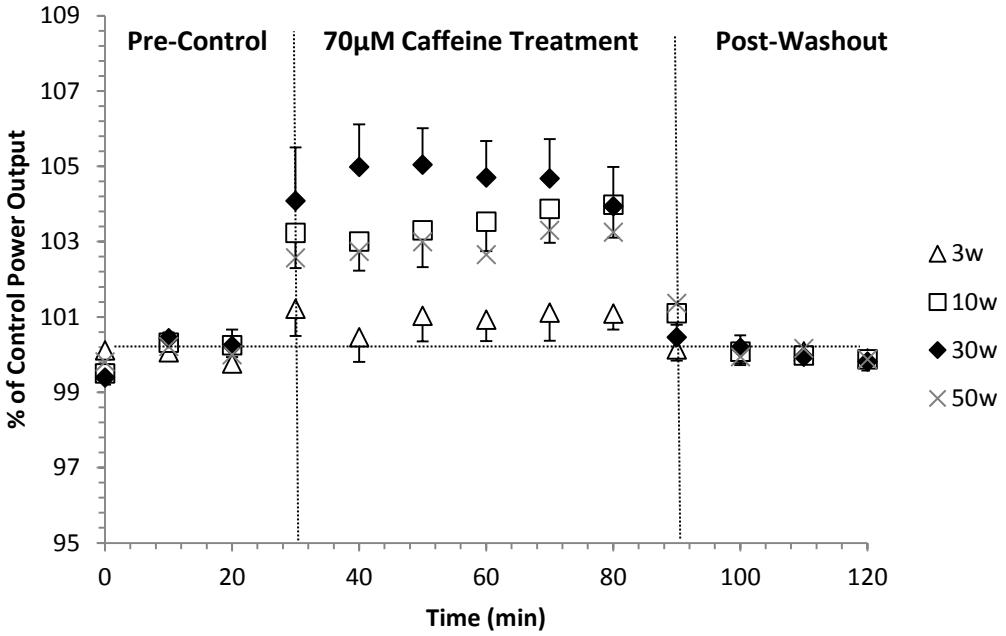
Figure Legends

Figure 1 – The effect of 70μM caffeine on the mean acute maximal power output of mouse EDL isolated from 3, 10, 30 and 50 week old mice [Data represented as mean ± SE: n=10 in each case).

Figure 2 – The effect of 70μM caffeine on the mean acute maximal power output of mouse diaphragm isolated from 3, 10, 30 and 50 week old mice [Data represented as mean ± SE: n=10 in each case).

Figure 3 - Comparison of the peak effect of caffeine on muscle power output, with increased age, between EDL and diaphragm [Each data point represented as mean ± SE: n=10 in each case].



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668 Figure 1

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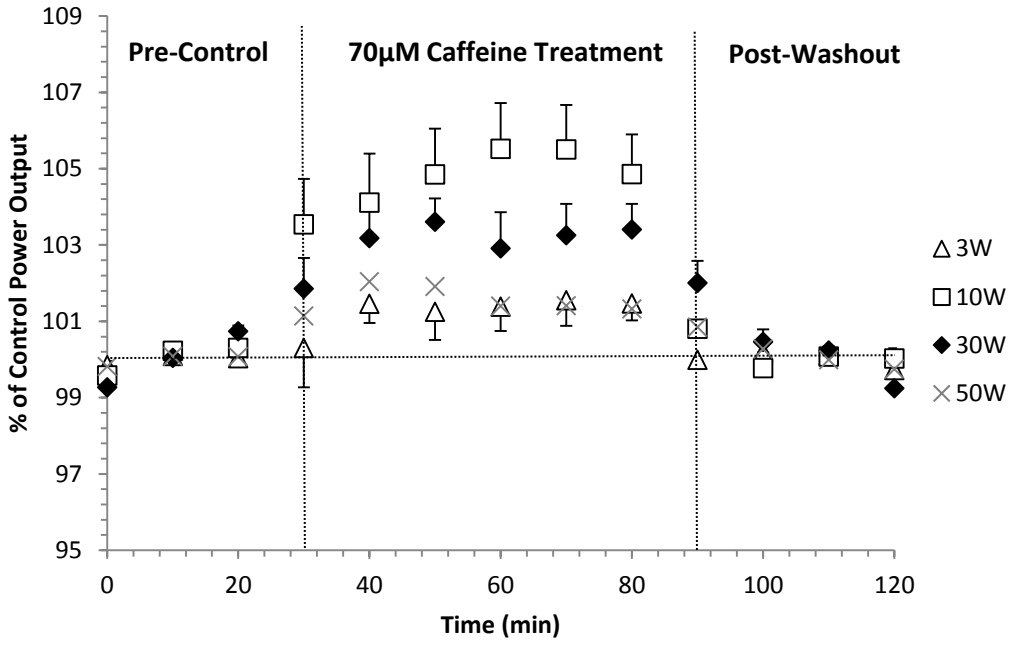
Pre-Control
70µM Caffeine Treatment
Post-Washout
% of Control Power Output
Time (min)
3W
10W
30W
50W
109
107
105
103
101
99
97
95
0
20
40
60
80
100
120

Figure 2

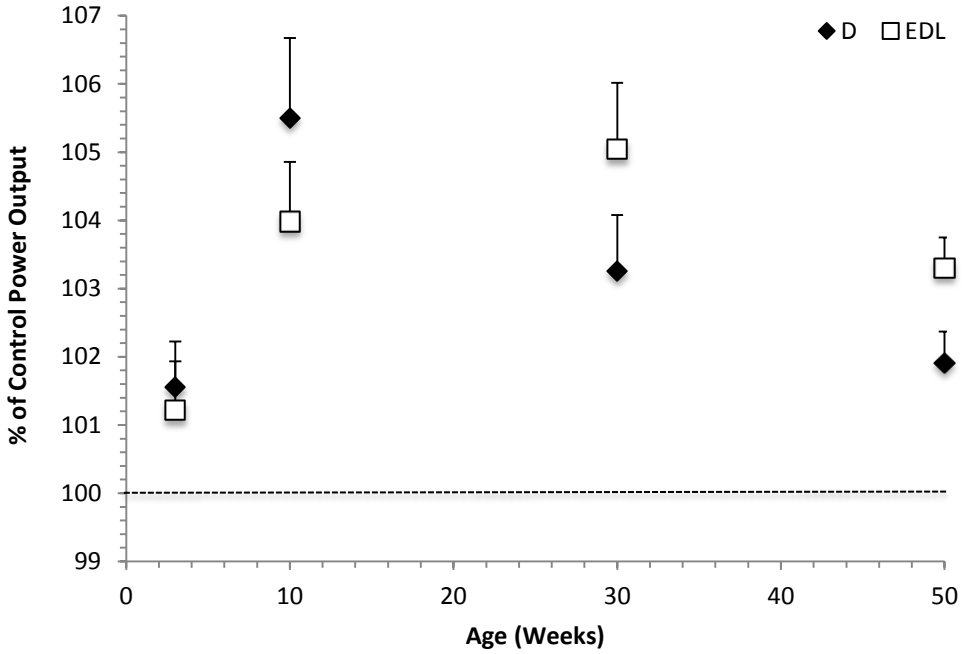
D
EDL
% of Control Power Output
107
106
105
104
103
102
101
100
99
0
10
20
30
40
50
Age (Weeks)

Figure 3

Table 1 – There was no relationship between caffeine treated peak power output and muscle mass

Animal	3 Wk Mmass (g)	3 Wk PPO (%)	10 Wk Mmass (g)	10 Wk PPO (%)	30 Wk Mmass (g)	30 Wk PPO (%)	50 Wk Mmass (g)	50 Wk PPO (%)
				EDL				
1	0.0077	103.15	0.0126	110.67	0.0137	103.96	0.018	103.96
2	0.0076	100.39	0.012	106.11	0.0136	109.21	0.0162	103.37
3	0.0099	101.79	0.0129	100.63	0.014	104.76	0.0165	101.75
4	0.0067	106.05	0.0133	107.89	0.0159	103.19	0.0152	103.78
5	0.0085	103.02	0.0132	102.76	0.0142	105.82	0.0214	104.64
6	0.0061	101.23	0.0111	105.12	0.0146	102.21	0.0155	104.06
7	0.0048	102.00	0.0127	102.96	0.0143	107.57	0.0133	104.85
8	0.0044	99.84	0.0098	102.59	0.0142	100.74	0.0137	101.44
9	0.0058	100.45	0.0141	105.70	0.0145	110.06	0.0167	103.28
10	0.0057	103.11	0.0127	102.93	0.0132	106.98	0.0134	105.48
r value		*0.233*		*0.168*		*-0.323*		*0.052*
P value		*0.516*		*0.642*		*0.362*		*0.887*
				Diaphragm				
1	0.0115	98.10	0.0176	111.52	0.0225	103.63	0.0369	101.80
2	0.0098	104.88	0.0157	102.35	0.0147	108.63	0.0308	101.86
3	0.0081	101.65	0.0187	101.65	0.015	102.48	0.0321	102.26
4	0.0078	103.58	0.0132	106.67	0.024	103.78	0.0226	103.24
5	0.0087	102.12	0.0131	106.20	0.0233	103.84	0.0298	98.78
6	0.005	102.80	0.012	112.02	0.0251	104.40	0.025	103.51
7	0.0071	101.14	0.0155	104.12	0.0264	103.99	0.0276	101.56
8	0.0047	103.89	0.016	107.38	0.0281	102.60	0.0203	104.24
9	0.0078	101.77	0.0159	104.95	0.0119	103.81	0.0264	101.77
10	0.0066	101.90	0.0171	101.80	0.0195	100.66	0.022	103.51
r value		*-0.465*		*-0.459*		*-0.231*		*0.593*
P value		*0.176*		*0.183*		*0.521*		*0.071*