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AN EXERCISE-INDUCED IMPROVEMENT IN ISOLATED SKELETAL MUSCLE CONTRACTILITY DOES NOT AFFECT THE PERFORMANCE-ENHANCING BENEFIT OF 70μM CAFFEINE TREATMENT

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SUMMARY STATEMENT

This study uniquely examines whether the performance-enhancing effect of caffeine is improved following exercise training by assessing the effects of 70μM caffeine on muscle isolated from trained and untrained mice.

ABSTRACT

This study aimed to examine the effects of exercise-induced increases in skeletal muscle contractile performance on isolated skeletal muscle caffeine sensitivity. 30-week old CD1 mice (n=28) either acted as controls or underwent eight weeks of voluntary wheel running. Following the treatment intervention, whole soleus (SOL) or a section of the costal diaphragm (DIA) was isolated from each mouse and tested to determine the effect of 70μM caffeine on work loop power output. Although caffeine elicited a significant increase in power of both the SOL and the DIA, relative to a non-caffeine control, the effect was not different between the experimental groups, despite the muscles of the trained group producing significantly greater muscle power. There was no significant relationship between training volume or baseline work loop power and the caffeine response. These results indicate that an exercise-induced increase in muscle performance did not influence the performance-enhancing effects of caffeine.

Key Words: Ergogenic Aid, Muscle Ageing, Work Loop, Muscle Power

INTRODUCTION

Despite a wealth of research documenting the performance-enhancing effects of caffeine on exercise performance in humans (see reviews (Graham, 2001, Astorino and Roberson, 2010), evidence is still contentious. One such area of uncertainty is whether caffeine elicits a greater performance-enhancing benefit in trained compared to untrained individuals. Systematic reviews assessing the effects of caffeine on high intensity exercise in humans indicate that well trained individuals are more likely to show a greater response to caffeine (Astorino and Roberson, 2010, Collomp et al., 1992). Mechanistically, caffeine has been shown to act centrally as an adenosine receptor antagonist promoting an elevated release of neurotransmitters and modulating perceptions of pain and fatigue (Davis and Green, 2009). Furthermore, there is evidence to suggest caffeine can act directly on skeletal muscle, at least in rodents and amphibians, to promote elevated power output (Tallis et al., 2015). What is not clear is to what extent the potential for an increased caffeine sensitivity in trained individuals is related to a greater ability to promote these mechanistic responses, or an improved ability of trained individuals to work maximally and consistently across numerous exercise bouts.

A recent review has highlighted the value of using an isolated skeletal muscle model to examine the potential performance-enhancing effects of caffeine (Tallis et al., 2015). Importantly, studies using isolated skeletal muscle allow the direct and muscle-specific effect of caffeine to be analysed, reduce the influence of caffeine habituation and inter-individual differences in caffeine digestion and distribution and, allow a highly repeatable measure of maximal muscle performance which is difficult to obtain *in vivo*.

Studies examining the direct effect of physiologically relevant (≤70 μM) concentrations of caffeine on isolated skeletal muscle in rodents have demonstrated a significant improvement in muscle power output, with a greater benefit in muscles with a higher composition of slower fibre type (Tallis et al., 2012, Tallis et al., 2013, James et al., 2005). 70μM caffeine has been used regularly in previous isolated muscle work to represent the maximal, non-toxic blood plasma concentration attainable in humans (James et al., 2005, Tallis et al., 2012, Tallis et al., 2017c). Such caffeine-induced improvements in muscle function have been attributed to the ability of caffeine to act as a direct adenosine receptor antagonist on adenosine A_1 receptors on the skeletal muscle membrane and/or bind to ryanodine receptors (RyR) resulting in altered intramuscular ion handling (Tallis et al., 2015). More specifically, caffeine treatment has been demonstrated to improve the opening of RyR2 channels resulting in greater Ca^{2+} release from the sarcoplasmic reticulum (SR), increase myofibrillar Ca^{2+} sensitivity, and reduce the activity of sarcoplasmic reticulum Ca^{2+}-ATPase (Allen and Westerblad, 1995, Magkos and Kavouras, 2005, Rossi et al., 2001). The net effect is greater basal and active intracellular Ca^{2+} concentration and, as a result, more rapid and numerous cross bridge formation.

We have recently shown that despite an early age-related decline in the contractile performance of isolated mouse skeletal muscle (i.e. within the first 50% of the animal lifespan, Tallis et al. (2014b)), direct treatment of 70μM caffeine was still effective in eliciting an improved muscle power output. However the magnitude of this performance-enhancing effect of caffeine was reduced when compared to muscle isolated from younger rodents (Tallis et al., 2017c). Given that the demonstrated reduction in contractile performance with age was primarily attributed to

dihydropyridine receptor-RYR uncoupling and a reduced Ca^{2+} availability at the contractile proteins, the age-related reduction in the caffeine response was attributed to a reduced ability to evoke elevated SR Ca^{2+} release. We have shown that such detrimental effects on intramuscular Ca^{2+} handling are likely reversed following an eight week voluntary wheel running intervention (Tallis et al., 2017a). The present work aims to examine the effects of an exercise-induced increase in contractile performance on skeletal muscle contractile responses to physiologically relevant 70μM caffeine. It is hypothesised that 70μM caffeine will elicit a greater increase in muscle power output of a trained group compared to an untrained control group indicating an increased sensitivity to the effects of caffeine on skeletal muscle Ca^{2+} kinetics.

METHOD

The use of animals in this study was approved by the ethics committee of Coventry University. Female CD1 mice (Charles River) were bred and kept in house at the host institute. All animals had *ad libitum* access to food (CRM(P); SDS/Dietex International Ltd) and water, and were kept in 12:12 light:dark cycles at 50% relative humidity. Previous work has indicated that the age-related decline in muscle function and caffeine sensitivity occurs around the age of 30 weeks in this mouse strain (Tallis et al., 2014b), and subsequently 30 weeks formed the target age for the exercise intervention. Up until 30 weeks animals were housed in groups of eight and were then separated into individual cages to form an exercise intervention group (N=14) and a control group (N=14). Each mouse in the exercise group had access to a running wheel (diameter = 15cm) for eight weeks, with the total number of revolutions recorded for each 24-hour period. For the duration of the intervention control animals were housed in identical conditions, but without access to running wheels.

Muscle Preparation and Assessment of Contractility

Animals were sacrificed by cervical dislocation in accordance with British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1 and one of either whole soleus (SOL) from the left hind limb (N=7 for exercise intervention group and N=7 for control group), or a ventral section of the costal diaphragm (DIA) (N=7 for exercise intervention group and N=7 for control group) were extracted and prepared for mechanical assessment as per our previous work (Tallis et al., 2014b, Tallis et al., 2017b).

We have previously examined the dose response effect of physiological doses of caffeine in young rodents (Tallis et al., 2012), and although caffeine doses lower than 70μM have elicited a direct muscle effect, we deemed it necessary for the first assessment examining training effects in an older rodent group to examine the maximal physiological dose. The experimental procedure for assessing the effect of 70μM caffeine on muscle contractility followed our previously published protocol (Tallis et al., 2012, James et al., 2005, Tallis et al., 2017b). Briefly, each muscle preparation was placed in a custom-designed muscle rig containing circulating oxygenated Krebs-Henseleit solution (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), which was maintained at a constant 37°C. Length and stimulation parameters were optimised to evoke maximal isometric twitch and tetanus responses. The work loop technique was then used, with length change strain and cycle frequency optimised to evoke maximal work loop power at 5Hz and 7Hz cycle frequency for SOL and DIA respectively. Previous evidence has demonstrated these cycle frequencies to be optimal for maximising net work loop power output in these muscles (Altringham and Young, 1991, James et al., 1995). Once net work loop power output was optimised, it was then monitored over 30 minutes before the Krebs solution was replaced with Krebs solution containing 70μM caffeine. Net work loop power output was then monitored for 60 minutes, before the caffeinated Krebs solution was replaced by the control Krebs solution for a 40-minute washout period.

Upon completion of the experimental procedure, the muscle was removed from the rig and the bone and/or tendon used to fix the muscle was removed. The remaining muscle

was then blotted on absorbent paper and wet muscle mass measured to the nearest 0.0001 g. Normalised muscle power output (W kg^{-1}) was calculated as net work loop power output divided by wet muscle mass.

Statistical Method

In the absence of vascular perfusion, the performance of the muscle will slowly deteriorate over time due to the development of an anoxic core (Barclay, 2005). Over the time course of the experiment (typically 130 minutes), the power producing capacity of the muscles was reduced by 6.9±1.1% which is similar in magnitude to that seen in previous studies using the same methodological approach (Higgins et al., 2013, Tallis et al., 2012, Tallis et al., 2017c, James et al., 2005). To prevent the deterioration in muscle power output masking the effect of caffeine on power, a 1^{st} order regression equation was calculated between the control data and the washout data to identify the linear relationship between muscle power output and time. The regression equation was then used to determine control muscle power output for each time point during caffeine treatment (i.e. the power that we predict would have been obtained at each time point if the muscle remained in standard Krebs solution). The difference between the recorded caffeine treated power and the predicted control power was used to determine a treatment effect (James et al., 2005, Tallis et al., 2012, Tallis et al., 2014a, Tallis et al., 2017c).

Using this corrected data, a paired T-Test was performed to assess difference in work loop power output between the pre-treatment control and post-treatment washout for each muscle. As there was no significant difference ($P>0.66$ in all cases) between pre-

treatment control and post-treatment washout data for each experimental group; all control data were used for each animal.

All percentage data were converted to a proportion of the highest power output value, at any time by any muscle, and then arcsine transformed to reduce heterogeneity of variance (Black, 1999). A general linear model was used to assess the effect of caffeine treatment and training on power output, using muscle preparation (a separate code for each muscle preparation) as a random factor. The data used for each muscle in this general linear model was the first 3 time points (pre-treatment), timepoints 10, 20, 30, 40 and 50 minutes after caffeine treatment began, and the final 3 timepoints of post treatment washout. A Pearson's correlation coefficient was determined to analyse the relationship between total distance covered (training volume) during the wheel running intervention and the caffeine-induced change in maximal work loop power output. Further correlation coefficients were determined between pre-caffeine treatment maximal work loop power output (a proxy for the effect of training) for both the SOL and DIA and the caffeine-induced change in maximal net work loop power output.

All statistical analysis was performed using SPSS (Version 22, SPSS) and significance was determined when $P<0.05$. Data is represented as mean ± SE.

RESULTS

Power output significantly increased in both SOL and DIA when exposed to 70µM caffeine (Fig 1a & 1b; $F_{1,138}$>48 P <0.001 for each muscle). However, the caffeine-induced increase in net work loop power output did not prove to be significantly different between the trained and control groups (Fig 1a & 1b; $F_{1,138}$>2.25 P>0.13 for each muscle).

When the greatest level of response during the treatment period was extracted, the results indicate that caffeine treatment improved power output of the SOL and DIA by up to 2.8% and 4.0% respectively (Fig 2). Neither pre-caffeine treatment maximal net work loop power output for either muscle, nor training volume significantly correlated with the magnitude of the caffeine response (Fig 3; Pearson's R = -0.47; 0.20; 0.31 for maximal DIA power, maximal SOL power and total wheel running distance respectively; P>0.09).

DISCUSSION

Despite a substantial training-induced increase in muscle power output of >25% for both muscles respectively (see (Tallis et al., 2017a) for a full account of the contractile changes induced by the running wheel intervention), eight weeks of voluntary wheel running failed to elicit a change in the caffeine-induced increase in muscle power when compared to untrained controls. These data demonstrate for the first time that, at the muscle level, the ergogenic (performance-enhancing) effect of caffeine cannot be improved by exercise training. We suggest that previous *in vivo* work that has indicated greater caffeine-induced high intensity performance benefits for highly trained athletes compared to less active counterparts (Collomp et al., 1992, Astorino and Roberson, 2010), is likely due to an improved ability to exercise to exhaustion and the reproducibility of maximal efforts due to other mechanisms than as a direct caffeine-induced improvement in skeletal muscle performance.

As per previous studies, the caffeine-induced increase in muscle power observed in the present study is likely to be attributed to the action of caffeine as a direct adenosine receptor antagonist and/or its ability to bind to RYR receptors promoting a greater efflux of Ca^{2+} into the muscle cytoplasm (Tallis et al., 2012, Allen and Westerblad, 1995). Our previous work has indicated that 30 week old female CD1 mice experience early onset ageing in the form of dynapenia (Tallis et al., 2014b), quantified as an age associated reduction in contractile function independent of a change in muscle mass (Clark and Manini, 2008). The age-related decline in muscle function substantially accelerates beyond 30 weeks of age and has been in part attributed to DHPR-RYR uncoupling (Renganathan et al., 1997, Tallis et al., 2014b). DHPR-RYR uncoupling

results in impaired muscle Ca^{2+} release from the SR, and as a result, reduced cross bridge formation. Evidence indicates that exercise training may promote improvements in the excitation contraction coupling process (Munkvik et al., 2010). Our previous work has indicated that this is likely the mechanism driving the exercise-induced increase in contractile performance following eight weeks of voluntary wheel running (Tallis et al., 2017a). Despite this, the present data indicates that this form of training may not induce sufficient DHPR-RYR re-coupling to promote greater caffeine sensitivity in older adults. Such findings may indicate an age associated limitation in muscle plasticity that occurs with training older muscle and as such may provide further evidence for inevitable age-related decline in muscle function with increasing age that occurs irrespective of an active life style.

These findings indicate that an exercise-induced increase in skeletal muscle contractility does not negate the previously observed age-related decline in the effectiveness of caffeine to elicit improved skeletal muscle power output (Tallis et al., 2017c). Despite this, if directly transferable to human skeletal muscle, these findings infer that caffeine could still be an effective acute nutritional supplement in evoking performance improvements in both sedentary and active populations. Given that the animals used in this study were in the early stage of muscle ageing, it possible that a further reduction in the ergogenicity of caffeine could occur at even older ages despite the maintenance of an active life style. Given suggestions that nutritional ergogenic aids may be a useful adjunctive therapy to enhance the effects of exercise in the elderly or to offset loss in physical function with age (Cherniack, 2012), the data presented here are a needed first step in understanding the effect of caffeine when combined with training in ageing muscle.

In order to further understand the relationship between caffeine and exercise training, future work should focus on examining the effect of exercise on 'other' mechanisms by which caffeine elicits a performance-enhancing effect. Caffeine is known to act as a central adenosine receptor antagonist, promoting an elevated release of neurotransmitters (Ribeiro and Sebastiao, 2010, Fredholm et al., 1999). It is not yet known if exercise training can mediate such effects. Furthermore, future work should also consider the effect of exercise on caffeine sensitivity across a range of ages. Currently there is no data to indicate how caffeine sensitivity is affected by more advanced ageing or the role of physical activity in mediating the caffeine response in both young and older individuals.

In conclusion, an exercise-induced increase in the contractile performance of skeletal muscle did not affect the direct effect of 70μM caffeine treatment on skeletal muscle during early aging. The proposal, in previous literature, of a greater caffeine-induced increase in performance in well trained human individuals is therefore likely related to an improved ability to reproduce maximal efforts via the effect of caffeine on the central nervous system.

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CONFLICT OF INTEREST

None.

FIGURES

Figure. 1. The acute effect of 70 µM caffeine on net work loop power output of trained and untrained mouse soleus (A) and diaphragm (B) muscle [Data represented as Mean±SE; n=7 in each case].

Figure. 2. The peak effect of 70 µM caffeine treatment on net work loop power output of trained and untrained mouse diaphragm muscle [Data represented as symbols for each individual and a dash to indicate the mean value; n=7 in each case].

Figure. 3. The relationship between soleus (A) and diaphragm (B) pre-caffeine treatment maximal net work loop power output, total distance covered during the voluntary wheel running protocol (C) and the caffeine-induced change (response) in maximal work loop power output [n=14 in each case].

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