

# An Exercise-Induced Improvement In Isolated Skeletal Muscle Contractility Does Not Affect The Performance-Enhancing Benefit Of 70 [Mu]M Caffeine Treatment.

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1 **AN EXERCISE-INDUCED IMPROVEMENT IN ISOLATED SKELETAL**  
2 **MUSCLE CONTRACTILITY DOES NOT AFFECT THE PERFORMANCE-**  
3 **ENHANCING BENEFIT OF 70 $\mu$ M CAFFEINE TREATMENT**

4

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15 **SUMMARY STATEMENT**

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17 This study uniquely examines whether the performance-enhancing effect of caffeine is  
18 improved following exercise training by assessing the effects of 70 $\mu$ M caffeine on  
19 muscle isolated from trained and untrained mice.

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26 **ABSTRACT**

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

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

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51 **INTRODUCTION**

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53 Despite a wealth of research documenting the performance-enhancing effects of  
54 caffeine on exercise performance in humans (see reviews (Graham, 2001, Astorino and  
55 Roberson, 2010), evidence is still contentious. One such area of uncertainty is whether  
56 caffeine elicits a greater performance-enhancing benefit in trained compared to  
57 untrained individuals. Systematic reviews assessing the effects of caffeine on high  
58 intensity exercise in humans indicate that well trained individuals are more likely to  
59 show a greater response to caffeine (Astorino and Roberson, 2010, Collomp et al.,  
60 1992). Mechanistically, caffeine has been shown to act centrally as an adenosine  
61 receptor antagonist promoting an elevated release of neurotransmitters and modulating  
62 perceptions of pain and fatigue (Davis and Green, 2009). Furthermore, there is evidence  
63 to suggest caffeine can act directly on skeletal muscle, at least in rodents and  
64 amphibians, to promote elevated power output (Tallis et al., 2015). What is not clear is  
65 to what extent the potential for an increased caffeine sensitivity in trained individuals  
66 is related to a greater ability to promote these mechanistic responses, or an improved  
67 ability of trained individuals to work maximally and consistently across numerous  
68 exercise bouts.

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

76 Studies examining the direct effect of physiologically relevant ( $\leq 70 \mu\text{M}$ ) concentrations  
77 of caffeine on isolated skeletal muscle in rodents have demonstrated a significant  
78 improvement in muscle power output, with a greater benefit in muscles with a higher  
79 composition of slower fibre type (Tallis et al., 2012, Tallis et al., 2013, James et al.,  
80 2005).  $70\mu\text{M}$  caffeine has been used regularly in previous isolated muscle work to  
81 represent the maximal, non-toxic blood plasma concentration attainable in humans  
82 (James et al., 2005, Tallis et al., 2012, Tallis et al., 2017c). Such caffeine-induced  
83 improvements in muscle function have been attributed to the ability of caffeine to act  
84 as a direct adenosine receptor antagonist on adenosine  $A_1$  receptors on the skeletal  
85 muscle membrane and/or bind to ryanodine receptors (RyR) resulting in altered  
86 intramuscular ion handling (Tallis et al., 2015). More specifically, caffeine treatment  
87 has been demonstrated to improve the opening of RyR2 channels resulting in greater  
88  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum (SR), increase myofibrillar  $\text{Ca}^{2+}$   
89 sensitivity, and reduce the activity of sarcoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase (Allen and  
90 Westerblad, 1995, Magkos and Kavouras, 2005, Rossi et al., 2001). The net effect is  
91 greater basal and active intracellular  $\text{Ca}^{2+}$  concentration and, as a result, more rapid and  
92 numerous cross bridge formation.

93

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

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

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126 **METHOD**

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128 The use of animals in this study was approved by the ethics committee of Coventry  
129 University. Female CD1 mice (Charles River) were bred and kept in house at the host  
130 institute. All animals had *ad libitum* access to food (CRM(P); SDS/Dietex International  
131 Ltd) and water, and were kept in 12:12 light:dark cycles at 50% relative humidity.  
132 Previous work has indicated that the age-related decline in muscle function and caffeine  
133 sensitivity occurs around the age of 30 weeks in this mouse strain (Tallis et al., 2014b),  
134 and subsequently 30 weeks formed the target age for the exercise intervention. Up until  
135 30 weeks animals were housed in groups of eight and were then separated into  
136 individual cages to form an exercise intervention group (N=14) and a control group  
137 (N=14). Each mouse in the exercise group had access to a running wheel (diameter =  
138 15cm) for eight weeks, with the total number of revolutions recorded for each 24-hour  
139 period. For the duration of the intervention control animals were housed in identical  
140 conditions, but without access to running wheels.

141

142 *Muscle Preparation and Assessment of Contractility*

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144 Animals were sacrificed by cervical dislocation in accordance with British Home Office  
145 Animals (Scientific Procedures) Act 1986, Schedule 1 and one of either whole soleus  
146 (SOL) from the left hind limb (N=7 for exercise intervention group and N=7 for control  
147 group), or a ventral section of the costal diaphragm (DIA) (N=7 for exercise  
148 intervention group and N=7 for control group) were extracted and prepared for  
149 mechanical assessment as per our previous work (Tallis et al., 2014b, Tallis et al.,  
150 2017b).

151

152 We have previously examined the dose response effect of physiological doses of  
153 caffeine in young rodents (Tallis et al., 2012), and although caffeine doses lower than  
154 70 $\mu$ M have elicited a direct muscle effect, we deemed it necessary for the first  
155 assessment examining training effects in an older rodent group to examine the maximal  
156 physiological dose. The experimental procedure for assessing the effect of 70 $\mu$ M  
157 caffeine on muscle contractility followed our previously published protocol (Tallis et  
158 al., 2012, James et al., 2005, Tallis et al., 2017b). Briefly, each muscle preparation was  
159 placed in a custom-designed muscle rig containing circulating oxygenated Krebs-  
160 Henseleit solution (composition (mM) NaCl 118; KCl 4.75; MgSO<sub>4</sub> 1.18; NaHCO<sub>3</sub>  
161 24.8; KH<sub>2</sub>PO<sub>4</sub> 1.18; glucose 10; CaCl<sub>2</sub> 2.54; pH 7.55 at room temperature prior to  
162 oxygenation), which was maintained at a constant 37°C. Length and stimulation  
163 parameters were optimised to evoke maximal isometric twitch and tetanus responses.  
164 The work loop technique was then used, with length change strain and cycle frequency  
165 optimised to evoke maximal work loop power at 5Hz and 7Hz cycle frequency for SOL  
166 and DIA respectively. Previous evidence has demonstrated these cycle frequencies to  
167 be optimal for maximising net work loop power output in these muscles (Altringham  
168 and Young, 1991, James et al., 1995). Once net work loop power output was optimised,  
169 it was then monitored over 30 minutes before the Krebs solution was replaced with  
170 Krebs solution containing 70 $\mu$ M caffeine. Net work loop power output was then  
171 monitored for 60 minutes, before the caffeinated Krebs solution was replaced by the  
172 control Krebs solution for a 40-minute washout period.

173

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



176 was then blotted on absorbent paper and wet muscle mass measured to the nearest  
177 0.0001 g. Normalised muscle power output ( $\text{W kg}^{-1}$ ) was calculated as net work loop  
178 power output divided by wet muscle mass.

179

#### 180 *Statistical Method*

181

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

197

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

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

203

204 All percentage data were converted to a proportion of the highest power output value,  
205 at any time by any muscle, and then arcsine transformed to reduce heterogeneity of  
206 variance (Black, 1999). A general linear model was used to assess the effect of caffeine  
207 treatment and training on power output, using muscle preparation (a separate code for  
208 each muscle preparation) as a random factor. The data used for each muscle in this  
209 general linear model was the first 3 time points (pre-treatment), timepoints 10, 20, 30,  
210 40 and 50 minutes after caffeine treatment began, and the final 3 timepoints of post  
211 treatment washout. A Pearson's correlation coefficient was determined to analyse the  
212 relationship between total distance covered (training volume) during the wheel running  
213 intervention and the caffeine-induced change in maximal work loop power output.  
214 Further correlation coefficients were determined between pre-caffeine treatment  
215 maximal work loop power output (a proxy for the effect of training) for both the SOL  
216 and DIA and the caffeine-induced change in maximal net work loop power output.

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

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226 **RESULTS**

227

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

233

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

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251 **DISCUSSION**

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253 Despite a substantial training-induced increase in muscle power output of >25% for  
254 both muscles respectively (see (Tallis et al., 2017a) for a full account of the contractile  
255 changes induced by the running wheel intervention), eight weeks of voluntary wheel  
256 running failed to elicit a change in the caffeine-induced increase in muscle power when  
257 compared to untrained controls. These data demonstrate for the first time that, at the  
258 muscle level, the ergogenic (performance-enhancing) effect of caffeine cannot be  
259 improved by exercise training. We suggest that previous *in vivo* work that has indicated  
260 greater caffeine-induced high intensity performance benefits for highly trained athletes  
261 compared to less active counterparts (Collomp et al., 1992, Astorino and Roberson,  
262 2010), is likely due to an improved ability to exercise to exhaustion and the  
263 reproducibility of maximal efforts due to other mechanisms than as a direct caffeine-  
264 induced improvement in skeletal muscle performance.

265

266 As per previous studies, the caffeine-induced increase in muscle power observed in the  
267 present study is likely to be attributed to the action of caffeine as a direct adenosine  
268 receptor antagonist and/or its ability to bind to RYR receptors promoting a greater  
269 efflux of  $\text{Ca}^{2+}$  into the muscle cytoplasm (Tallis et al., 2012, Allen and Westerblad,  
270 1995). Our previous work has indicated that 30 week old female CD1 mice experience  
271 early onset ageing in the form of dynapenia (Tallis et al., 2014b), quantified as an age  
272 associated reduction in contractile function independent of a change in muscle mass  
273 (Clark and Manini, 2008). The age-related decline in muscle function substantially  
274 accelerates beyond 30 weeks of age and has been in part attributed to DHPR-RYR  
275 uncoupling (Renganathan et al., 1997, Tallis et al., 2014b). DHPR-RYR uncoupling

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

287

288 These findings indicate that an exercise-induced increase in skeletal muscle  
289 contractility does not negate the previously observed age-related decline in the  
290 effectiveness of caffeine to elicit improved skeletal muscle power output (Tallis et al.,  
291 2017c). Despite this, if directly transferable to human skeletal muscle, these findings  
292 infer that caffeine could still be an effective acute nutritional supplement in evoking  
293 performance improvements in both sedentary and active populations. Given that the  
294 animals used in this study were in the early stage of muscle ageing, it possible that a  
295 further reduction in the ergogenicity of caffeine could occur at even older ages despite  
296 the maintenance of an active life style. Given suggestions that nutritional ergogenic aids  
297 may be a useful adjunctive therapy to enhance the effects of exercise in the elderly or  
298 to offset loss in physical function with age (Cherniack, 2012), the data presented here  
299 are a needed first step in understanding the effect of caffeine when combined with  
300 training in ageing muscle.

301

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

312

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

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351 **CONFLICT OF INTEREST**

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376 **FIGURES**

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

381

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

385

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

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