

# Regional thermal specialisation in a mammal: temperature affects power output of core muscle more than that of peripheral muscle in adult mice (*Mus musculus*)

James, R.S. , Tallis, J. and Angilletta, M.J.

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1 **Regional thermal specialisation in a mammal: temperature affects power output of**  
2 **core muscle more than that of peripheral muscle in adult mice (*Mus musculus*)**

3

4 Rob S. James · Jason Tallis · Michael J. Angilletta Jr

5

6 **Abstract** In endotherms, such as mammals and birds, internal organs can specialize  
7 to function within a narrow thermal range. Consequently, these organs should become  
8 more sensitive to changes in body temperature. Yet, organs at the periphery of the body  
9 still experience considerable fluctuations in temperature, which could select for lower  
10 thermal sensitivity. We hypothesised that the performance of soleus muscle taken from  
11 the leg would depend less on temperature than would the performance of diaphragm  
12 muscle taken from the body core. Soleus and diaphragm muscles were isolated from mice  
13 and subjected to isometric and work-loop studies to analyse mechanical performance at  
14 temperatures between 15 °C and 40 °C. Across this thermal range, soleus muscle took  
15 longer to generate isometric force and longer to relax, and tended to produce greater  
16 normalised maximal force (stress) than did diaphragm muscle. The time required to  
17 produce half of maximal force during isometric tetanus and the time required to relax to  
18 half of maximal force were both more sensitive to temperature in soleus than they were in  
19 diaphragm. However, thermal sensitivities of maximal force during isometric tetani were  
20 similar for both muscles. Consistent with our hypothesis, power output (the product of  
21 speed and force) was greater in magnitude and more thermally sensitive in diaphragm  
22 than it was in soleus. Our findings, when combined with previous observations of  
23 muscles from regionally endothermic fish, suggest that endothermy influences the  
24 thermal sensitivities of power output in core and peripheral muscles.

25

26 **Keywords** endotherm · force · power · temperature · tetanus · thermal sensitivity

27

28 R. S. James (corresponding author) · J. Tallis

29 Department of Applied Sciences and Health, Coventry University, Coventry, CV1 5FB,

30 UK

31 e-mail: r.james@coventry.ac.uk

32 Tel: 024 7679 5803 Fax: 024 7688 8778

33

34 M. J. Angilletta Jr

35 School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA

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37

## 38 **Introduction**

39

40 The strategies animals adopt for coping with variation in temperature can be mapped  
41 against two continuous dimensions, namely thermal sensitivity (specialist to generalist)  
42 and thermoregulation (thermoconforming to thermoregulating) (Angilletta 2009). A  
43 thermal specialist has higher thermal sensitivity but higher peak performance than a  
44 thermal generalist, enabling it to perform relatively well over a relatively narrow range of  
45 temperatures. Endotherms thermoregulate, normally maintaining relatively high and  
46 constant body temperature compared to ectotherms. Theory predicts that a key benefit of  
47 such precise thermoregulation in endotherms is that physiological processes will be  
48 enhanced via high thermal specialisation, leading to high thermal sensitivity (Angilletta et  
49 al. 2010). Many endotherms are homeothermic, tightly regulating their core temperature  
50 within a range of less than 3°C (Refinetti 1999; Wooden and Walsberg 2004). However,  
51 peripheral muscles of endotherms can still endure much variation in temperature; for  
52 example, some peripheral muscles of humans undergo changes of more than 15°C as  
53 their environment warms or cools (Ducharme et al. 1991; Ranatunga 1998). Even large  
54 peripheral muscles undergo appreciable temperature changes. During exercise in  
55 controlled environments, peripheral muscles of humans warm by 3° to 4°C (Saltin et al.  
56 1968; Kenny et al. 2003; Castle et al. 2006; Yaicharoen et al. 2012). Importantly, changes  
57 in air temperature could exacerbate changes caused by physiological states such as  
58 exercise.

59 The temperature of a muscle has profound effects on its contractile performance. As a  
60 muscle warms, it produces force more rapidly, shortens and relaxes more quickly, and  
61 achieves a greater peak force (Bennett 1984; Rall and Woledge 1990; Marsh 1994; Syme  
62 2006; James 2013). These changes in the intrinsic properties of muscle lead to greater  
63 power output at higher temperatures, as long as temperature does not get too high (Rome  
64 and Swank 1992; Swoap et al. 1993; Herrel et al. 2007; James et al. 2012). Recent  
65 findings also indicate that warmer muscles use less energy to produce power, possibly  
66 due to a reduction in passive stiffness (Seebacher et al. 2014). Endothermic  
67 thermoregulation enables mammals and birds to maintain warm bodies and enhance  
68 performance even when the environment cools. Indeed, muscular and locomotor

69 performances are usually maximised at the set-point temperature (James 2013). All  
70 endotherms thermoregulate regionally since they can either defend core temperature or  
71 surface temperature, but not both (Lovegrove et al. 1991). For instance, tuna and lamnid  
72 sharks have an endothermic core that keeps interior muscles at a higher temperature than  
73 superficial ones, yielding more power and faster swimming. Skeletal muscle from the  
74 warm core produces high power over a narrow range of temperatures, which exceed sea  
75 temperatures. In fact, slow fibres from the endothermic core produce greater peak power  
76 but are more sensitive to temperature than either superficial muscles from the same  
77 species or core muscles from ectothermic species (Altringham and Block 1997; Bernal et  
78 al. 2005; Donley et al. 2007; Donley et al. 2012). These findings indicate a specialist-  
79 generalist trade-off that constrains the evolution of skeletal muscle.

80 Because endotherms regulate their core temperatures more tightly than their peripheral  
81 temperatures, muscle from the core should be more specialized. Thus, we predict that  
82 core muscles will have greater peak performance but will be more sensitive to  
83 temperature than peripheral muscles. Whilst thermal sensitivities of muscular and  
84 locomotor performances of ectotherms have been studied extensively, we know very little  
85 about thermal sensitivities of these performances in endotherms. Moreover, to our  
86 knowledge, no one has published a comparison of the thermal sensitivities of mammalian  
87 muscle from the core with mammalian muscle from the periphery (Angilletta et al. 2010;  
88 James 2013). Thus, we aimed to compare thermal sensitivities of performances by  
89 diaphragm muscle (core) and soleus muscle (periphery) in mice. For both types of  
90 muscle, we measured isometric (constant length) and work-loop performance (power  
91 production during length change cycles). Based on our hypothesis, we expected two  
92 patterns to emerge from our comparisons of these muscles within individuals. First, we  
93 expected diaphragm muscle to produce greater power at the core temperature than would  
94 soleus muscle. Second, we expected the performance of diaphragm muscle to depend  
95 more on temperature than would the performance of soleus muscle.

96

97

## 98 **Materials and methods**

99

100

101 Tissue samples

102

103 Mice (*Mus musculus*, Linnaeus 1758; strain CD1, Charles River, UK) were bred and  
104 maintained at Coventry University at 19 to 22 °C. Adult mice ( $n=8$ ; body mass =  
105  $35.2\pm 0.9$  g mean  $\pm$  s.e.m.) were euthanased by dislocation of the neck in accordance with  
106 British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1. Body mass  
107 was determined to the nearest 0.1 g using an electronic balance. One hind-limb was  
108 removed and soleus muscle was rapidly isolated in chilled (4-6 °C), oxygenated (95%  
109 O<sub>2</sub>; 5% CO<sub>2</sub>) Krebs-Henseleit solution (composition, values in mM: NaCl 118; KCl  
110 4.75; MgSO<sub>4</sub> 1.18; NaHCO<sub>3</sub> 24.8; KH<sub>2</sub>PO<sub>4</sub> 1.18; glucose 10; CaCl<sub>2</sub> 2.54; pH 7.55 at  
111 room temperature prior to oxygenation). A piece of bone was left at the end of both the  
112 proximal and distal tendons of soleus and aluminium foil clips were placed around the  
113 tendons. Meanwhile a ventral section of the costal diaphragm was removed whilst kept in  
114 Krebs as described above. A rib and part of the central tendon were left attached to the  
115 diaphragm preparation. An aluminium foil T-clip was placed around the central tendon of  
116 the diaphragm. The methods used for isometric and work-loop studies are based on those  
117 used in previous studies (Seebacher and James 2008; James et al. 2012).

118

119

120 Isometric studies

121

122 Isometric studies were used to determine the twitch and tetanus kinetics of isolated  
123 muscle. The bone or foil clip at one end of the muscle preparation was clamped via a  
124 crocodile clip to a strain gauge (UF1, Pioden Controls Ltd, Canterbury, Kent, UK),  
125 whereas the bone or foil clip at the other end was clamped via a crocodile clip to a motor  
126 arm (V201, Ling Dynamics Systems, Royston, Herts, UK) attached to an LVDT (Linear  
127 Variable Displacement Transformer, DFG 5.0, Solartron Metrology, Bognor Regis,  
128 Sussex, UK). The LVDT was used to monitor the length changes delivered to the muscle

129 preparation. The whole of the muscle, tendon and bone preparation was then allowed to  
130 equilibrate within the organ bath at the first test temperature for 10 to 15 minutes in  
131 circulating, oxygenated (95% O<sub>2</sub>; 5% CO<sub>2</sub>) Kreb's solution. The preparation was then  
132 held at constant length and stimulated via parallel platinum electrodes to deliver square  
133 wave stimuli of 2 ms pulse width that generated a series of twitches. Stimulus amplitude  
134 and muscle length were adjusted to determine maximal isometric twitch force. An  
135 isometric tetanic force response was elicited by subjecting the diaphragm muscle to a 220  
136 ms train of stimulation and soleus to a 350 ms train of stimulation. Stimulation frequency  
137 was altered (140 to 160 Hz for diaphragm muscle; 120 to 140 Hz for soleus muscle) to  
138 determine maximal tetanic force. Time to half of maximal force during tetanus and time  
139 from last stimulus to half tetanus relaxation were measured. A rest period of 5 minutes  
140 was allowed between each tetanic response.

141

142

143 Work-loop analysis

144

145 The work-loop technique was used to determine the power output of muscles during  
146 cyclical length changes (Josephson 1985). Unlike fixed-length isometric studies and  
147 fixed-load isotonic studies of muscle performance, the work-loop technique allows  
148 measurement of muscle power output under length and activation changes that are  
149 generally more indicative of *in vivo* contractile performance (James et al. 1996; Caiozzo  
150 2002). Each muscle preparation was subjected to a set of four sinusoidal length changes  
151 symmetrical around the length found to generate maximal twitch force. The muscle was  
152 stimulated using the stimulation amplitude and stimulation frequency found to yield  
153 maximal isometric force. Electrical stimulation and length changes were controlled via a  
154 data acquisition board (KUSB3116, Keithley Instruments, Cleveland, OH, USA) and a  
155 custom-designed program developed with TestPoint software (CEC Testpoint version 7,  
156 Measurement Computing, Norton, MA, USA). Muscle force was plotted against muscle  
157 length for each cycle to generate a work-loop, the area of which equated to the net work  
158 produced by the muscle during the cycle of length change (Josephson 1985).  
159 Instantaneous power output was calculated for every data point in each work-loop (1,000

160 data points per work-loop) by multiplying instantaneous velocity by instantaneous force.  
161 Instantaneous power output values were averaged to generate an average power output for  
162 each length change cycle. The cycle frequency of length change was altered to determine  
163 the cycle frequency for maximal power output. Muscle strain was kept at 0.10 (10% peak  
164 to peak) of muscle fibre length for soleus (James et al. 1995), 0.13 of muscle fibre length  
165 for diaphragm (Altringham and Young 1991) at each cycle frequency as these strains  
166 have previously been found to yield maximal power output and fixing strain but varying  
167 cycle frequency simplified the procedure used. Every 5 minutes the muscle was subjected  
168 to a further set of four work-loop cycles with cycle frequency, stimulation duration and  
169 stimulation phase parameters being altered in between each set until maximum power  
170 output was determined.

171 After maximal power output was determined the temperature of the Krebs's solution  
172 bathing the muscle was altered to a new temperature over 10 to 20 minutes, allowing at  
173 least a further 10 minutes for the muscle to equilibrate to the new temperature. The  
174 isometric and work-loop studies were then repeated at the new temperature. Each muscle  
175 was subjected to four different temperatures and then the first temperature was repeated  
176 twice as a control for time (i.e., 1, 2, 3, 1, 4, 1). To maximize our power to compare  
177 thermal sensitivities, we randomly selected test temperatures from a continuous  
178 distribution between 15 and 40 °C (Steury and Murray 2005). A set of control parameters  
179 for sinusoidal length change and stimulation were imposed on the muscle every three to  
180 five sets of work-loops, whenever the muscle was at temperature 1, to monitor variation  
181 in the muscles ability to produce power over the time-course of the experiment. There  
182 were significant changes in absolute muscle power output over the time-course of the  
183 experiments ( $P=0.0074$ ; Table 3). Determination of the effects of power output on muscle  
184 performance typically took 4.5 hours per muscle, during which time diaphragm and  
185 soleus muscle power output typically decreased by about 6%, with no significant  
186 difference in the effect of time between muscles. Any variation in power was found to be  
187 due to a matching change in ability to produce force. Therefore, the power produced by  
188 each preparation was corrected to the control run at temperature 1 that yielded the highest  
189 power output, assuming that alterations in power generating ability were linear over time  
190 between the control runs delivered at temperature 1.



191 At the end of the isometric and work-loop experiments, the bones, foil clips and  
192 tendons were removed and each muscle was blotted on absorbent paper to remove excess  
193 Krebs's solution. Wet muscle mass was determined to the nearest 0.0001 g using an  
194 electronic balance (Mettler-Toledo B204-S, Greifensee, Switzerland). Mean muscle  
195 cross-sectional area was calculated from muscle length and mass assuming a density of  
196  $1060 \text{ kg m}^{-3}$  (Mendez and Keys 1960). Maximum isometric muscle stress at each test  
197 temperature was then calculated as maximal tetanic force divided by mean cross-sectional  
198 area ( $\text{kN m}^{-2}$ ). Normalised muscle power output at each test temperature was calculated as  
199 power output divided by wet muscle mass ( $\text{W kg}^{-1}$ ).

200

201

202 Statistical modelling

203

204 We modelled the thermal sensitivity of four muscle performances: time to half of  
205 maximal force during tetanus; time to half tetanus relaxation, maximal absolute force;  
206 maximal absolute power. In each model, temperature and cross-sectional area of the  
207 muscle (or muscle mass) were treated as continuous independent variables and muscle  
208 type (diaphragm versus soleus) was considered a fixed factor. Cross-sectional area was  
209 used as an independent variable for force measurements, whereas mass was used as the  
210 independent morphometric variable for power measurements as force production is  
211 highly dependent on muscle cross-sectional area and power production is highly  
212 dependent on muscle mass. Since each preparation of muscle was tested repeatedly at  
213 different temperatures, we included time as a fixed factor to account for possible effects  
214 of fatigue or other form of time dependent deterioration of muscle. Multiple model types  
215 were tested for each muscle performance measure. The Akaike Information Criterion  
216 (AIC) was used to determine the best, most likely, model for each muscle performance.  
217 We started with the maximal model and then eliminated terms from the model, starting  
218 with the highest order term, until we arrived at the model with the lowest AIC (Crawley  
219 2007). All models were fit using the R Statistical Package (R Development Core Team  
220 2011).

221 A Cox proportional hazards model was fit to data for the time to half of maximal force

222 during tetanus and another such model was fit to data for time to half tetanus relaxation.  
223 Often used to fit data on survival, a proportional hazards model relates the time until  
224 some event occurs to a set of independent variables. Unlike parametric survival models,  
225 the nonparametric proportional hazards model makes few assumptions about the  
226 distribution of residuals. Because the responses of each muscle preparation throughout  
227 the experiment were likely correlated, we also included a robust sandwich estimator of  
228 the variance attributable to this random factor. Parameters were estimated using R's  
229 survival library (Therneau and Lumley 2009). Both the model for time to half of maximal  
230 force during tetanus and the model for time to half tetanus relaxation that had the lowest  
231 AIC included the terms muscle type (i.e. diaphragm versus soleus), and temperature.

232 A General Additive Model was fit to data for absolute force production. An additive  
233 model enabled us to estimate a nonlinear response to temperature, without knowing the  
234 form of the nonlinear function in advance (Zuur et al. 2009). Consequently, we preferred  
235 this approach to those that assume an exponential, an asymptotic, or a piecewise function  
236 (e.g., Arrhenius breakpoints). To avoid pseudoreplication, the identity of each muscle  
237 preparation was included as a random factor. Parameters were estimated using the *mgcv*  
238 (Wood 2004) and *nlme* (Pinheiro et al. 2011) libraries. The model for absolute force  
239 production that had the lowest AIC included the terms muscle cross-sectional area,  
240 muscle type (i.e. diaphragm versus soleus) and temperature.

241 A General Linear Model was fit to data for absolute power production. As in our  
242 statistical analysis of absolute force production, the identity of each muscle preparation  
243 was included as a random factor. Parameters were estimated using the *nlme* library  
244 (Pinheiro et al. 2011). The model for power production that had the lowest AIC included  
245 the terms muscle mass, muscle type (i.e. diaphragm versus soleus), temperature and time.  
246

**247 Results**

248

249 The best model for time to half of maximal force during tetanus and time to half tetanus  
250 relaxation described the vast majority of the variations in these traits (95% and 90%,  
251 respectively; Table 1) and included muscle type (diaphragm versus soleus), temperature  
252 and the interaction between muscle type and temperature. Both of these tetanus times  
253 decreased with increasing temperature in diaphragm and soleus muscles (Fig. 1, 2;  
254  $P<0.001$  in each case), but the thermal effects were greater in soleus muscle as indicated  
255 by significant interactions between muscle type and test temperature ( $P<0.007$  in each  
256 case; Table 1). Soleus muscle force took longer to relax than did diaphragm muscle  
257 ( $P=0.007$ ; Table 1; Fig. 2), but there was no significant difference in time to half of  
258 maximal tetanus force between muscles ( $P=0.52$ ).

259 The best model of maximal absolute isometric tetanic force described 86% of the  
260 variation, including effects of temperature, cross sectional area, muscle type, and the  
261 interaction between cross-sectional area and muscle type (Table 2). At all temperatures,  
262 soleus muscle tended to produce greater isometric tetanic force ( $P=0.072$ ) and tetanic  
263 stress (Fig. 3) than did diaphragm muscle. Since the best model excluded an interaction  
264 between muscle type and test temperature, thermal effects on isometric force are  
265 probably similar for soleus and diaphragm muscles. Maximal isometric force increased  
266 with a rise in temperature ( $P<0.0001$ ).

267 When we adjusted for muscle mass, the maximal net power generated during a work-  
268 loop increased with increasing temperature in diaphragm and soleus muscles (Fig. 4). At  
269 higher temperature power output was optimised at higher length change cycle frequency,  
270 yet still produced larger work-loops (Fig. 5). Diaphragm muscle produced greater net  
271 work-loop power than did soleus muscle on an absolute scale ( $P=0.006$ ) and relative to  
272 muscle mass (Fig. 4). Absolute muscle power output was significantly more thermally  
273 sensitive in diaphragm than it was in soleus (Table 3; Muscle type x Temperature  
274  $P<0.0001$ ). The thermal sensitivity of absolute muscle power output significantly  
275 increased with increased muscle mass in soleus and diaphragm muscles (Table 3;  
276  $P<0.0001$ ).

277

**278 Discussion**

279

280 We hypothesised that a muscle from the core of a mammalian body would be more  
281 sensitive to temperature than would a muscle from the periphery. This hypothesis follows  
282 from the premise that no organism can regulate its core and surface temperatures at the  
283 same time (Lovegrove et al. 1991). Since endotherms regulate core temperatures,  
284 peripheral temperatures will still fluctuate according to environmental conditions. A  
285 muscle in the extremities, such as the soleus, will experience even greater fluctuations in  
286 temperature than will a muscle at the periphery of the torso. In a given environment, the  
287 disparity between the thermal variances of core and peripheral muscles will increase as  
288 thermoregulation within the core becomes more precise.

289 To test our hypothesis, we compared the thermal sensitivities of diaphragm (core) and  
290 soleus (peripheral) muscles of mice. Both types of muscles were affected by temperature  
291 in ways that resembled thermal sensitivities previously reported for skeletal muscles of  
292 other species, including endotherms and ectotherms (Ranatunga 1982; Bennett 1984; Rall  
293 and Woledge 1990; Rome and Swank 1992; Swoap et al. 1993; Altringham and Block  
294 1997; De Ruiter et al. 1999; Herrel et al. 2007; Donley et al. 2012; James et al. 2012;  
295 James 2013). Muscles produced force more quickly, relaxed more quickly, and generated  
296 more power at higher temperatures. Presumably, the thermal optima for power output  
297 exceeds 40 °C in the muscles that we tested. Maximal activity of enzymes have also been  
298 found to occur at temperatures that exceed body temperatures (Bernal et al. 2003). These  
299 findings suggest that natural selection optimizes rather than maximises performance at  
300 body temperature. A few studies have considered how variables affecting power output,  
301 such as length and stimulation parameters, are differentially optimised for power output  
302 or efficiency (Curtin and Woledge 1993a; Curtin and Woledge 1993b), but this work has  
303 not been extended to consider the effects of temperature. Such studies would help us to  
304 better understand the relationship between a muscle's temperature and its performance.

305 Although warming generally enhanced muscle performance, thermal sensitivities of  
306 power output differed between soleus and diaphragm muscles. Consistent with our  
307 predictions, the power generated by diaphragm muscle was higher at core body  
308 temperature and changed more dramatically during warming than did the power

309 generated by soleus muscle. The difference in thermal sensitivity between the soleus and  
310 diaphragm muscles of mice accords with differences between muscles of regionally  
311 endothermic fish. Power generated by core muscles of tuna was more sensitive to  
312 warming than was power generated by peripheral muscles (Altringham and Block 1997).  
313 Similar differences in thermal sensitivity have also been found when comparing  
314 ectothermic and endothermic species (Rall and Woledge 1990; Choi et al. 1998; James  
315 2013). For instance, temperature affects the power generated by red muscle of regionally  
316 endothermic sharks more than it affects core muscles of ectothermic sharks (Donley et al.  
317 2007; Donley et al. 2012). Collectively, these results support the idea that endothermy  
318 imposes divergent selective pressures on core and peripheral muscles.

319 The thermal sensitivity of power output depends on the thermal sensitivities of  
320 contractile properties such as passive stiffness, force generation, and velocities of  
321 shortening and lengthening (James 2013). In endothermic fishes, both power output and  
322 isometric force generation of core muscle were more sensitive to temperature than were  
323 the same properties of peripheral muscle (Bernal et al. 2005; Donley et al. 2007; Donley  
324 et al. 2012). We observed no statistically significant differences between the thermal  
325 sensitivities of maximal isometric force of diaphragm and soleus muscles. However,  
326 diaphragm muscles relaxed faster than did soleus muscles, probably enabling diaphragm  
327 to undergo more frequent cycles of shortening and lengthening to generate higher  
328 maximal power output. Greater diaphragm muscle power output at higher temperatures  
329 stemmed from faster changes in length and higher force during shortening (Fig. 5). Both  
330 of these properties infer an increase in the maximal shortening velocity of the muscle.  
331 Therefore, any difference in thermal sensitivities of maximal shortening velocity between  
332 soleus and diaphragm could explain the observed thermal sensitivities of power output.  
333 Contrary to our expectation, temperature had a greater effect on the time to half maximal  
334 tetanus force in soleus than in diaphragm; however, since soleus produces maximal  
335 power at lower cycle frequencies, variation in the time required to produce force should  
336 only weakly influence net power output in soleus (James et al. 1996).

337 In conclusion, we provide the first evidence that thermal sensitivities differ between  
338 muscles within a mammal. Consistent with our hypotheses, power output was greater in  
339 magnitude and more sensitive to temperature in diaphragm than it was in soleus. When

340 combined with previous studies on regionally endothermic fish, our findings suggest that  
341 temperature affects the power production of an endotherm's skeletal muscle more in the  
342 core of the body than in the periphery. This finding has important implications for  
343 thermal adaptation in endotherms, which might have to choose between a muscle  
344 specialized to perform at the mean body temperature or a muscle that performs well over  
345 a broader range of temperature (Angilletta et al. 2010). Given the initial support for this  
346 trade-off, researchers should compare thermal sensitivities of core and peripheral muscles  
347 in a wider range of mammals, including those that frequently undergo torpor. These  
348 studies should also examine a broader set of contractile properties and muscle types.  
349 Replicating these comparisons among species and among muscles within species will  
350 enable researchers to determine whether differences in the thermal physiology of muscle  
351 stem from thermal adaptation rather than potentially confounding factors, such as fibre  
352 type distribution.

353

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472 **Fig. 1** Time to half of maximal force during tetanus decreased with increased test  
473 temperature in diaphragm and soleus muscles from mice ( $P<0.001$ ). Each symbol  
474 represents a measurement made on one individual.  $n=8$  muscles were used for both soleus  
475 and diaphragm. Each muscle was subjected to four different test temperatures.

476 **Fig. 2** Time to half tetanus relaxation decreased with increased test temperature in  
477 diaphragm and soleus muscles from mice ( $P<0.001$ ). Each symbol represents a  
478 measurement made on one individual.  $n=8$  muscles were used for both soleus and  
479 diaphragm. Each muscle was subjected to four different test temperatures.

480 **Fig. 3** Maximal isometric tetanic stress (force normalised to muscle cross-sectional area)  
481 increased with increased test temperature in diaphragm and soleus muscles from mice.  
482 Each symbol represents a measurement made on one individual.  $n=8$  muscles were used  
483 for both soleus and diaphragm. Each muscle was subjected to four different test  
484 temperatures.

485 **Fig. 4** Maximal work-loop net power output, normalised to muscle mass, increased with  
486 increased test temperature in diaphragm and soleus muscles from mice. Each symbol  
487 represents a measurement made on one individual.  $n=8$  muscles were used for both soleus  
488 and diaphragm. Each muscle was subjected to four different test temperatures.

489 **Fig. 5** A) Diaphragm work-loop shapes that generated maximal power output at 17 °C  
490 (broken line) and 37.7 °C (solid line) in the same muscle preparation. Maximal power  
491 output was produced at a length change cycle frequency of 2 Hz at 17 °C and 7 Hz at  
492 37.7 °C; B) Soleus work-loop shapes that generated maximal power output at 15.3 °C  
493 (broken line) and 37.4 °C (solid line) in the same muscle preparation. Maximal power  
494 output was produced at a length change cycle frequency of 1 Hz at 15.3 °C and 5 Hz at  
495 37.4 °C.

496 **Table 1** Parameters of the Cox proportional hazards models of time to half of maximal  
 497 force during tetanus and time to half tetanus relaxation. The best model included the  
 498 terms muscle type (i.e. diaphragm versus soleus), and temperature.

Effect	Coefficient	Robust SE	z	P
<i>T to half of maximal tetanus force:</i>				
Muscle type	0.68	1.07	0.64	0.52
Temperature	0.54	0.08	6.66	<0.001
Muscle type x Temperature	-0.14	0.05	-2.87	0.004
<i>T to half tetanus relaxation :</i>				
Muscle type	-2.63	0.98	-2.69	0.007
Temperature	0.75	0.08	9.21	<0.001
Muscle type x Temperature	-0.13	0.05	-2.72	0.006

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502 **Table 2** A General Additive Model of the effects of temperature, muscle cross-sectional  
503 area and muscle type (i.e. diaphragm versus soleus) on absolute force production.

Effect	df	<i>F</i>	<i>P</i>
Muscle cross-sectional area	1,58	27.1	<0.0001
Muscle type	1,58	3.38	0.0716
Temperature	3,6,58	83.3	<0.0001
Muscle area x Muscle type	1,58	10.8	0.0017

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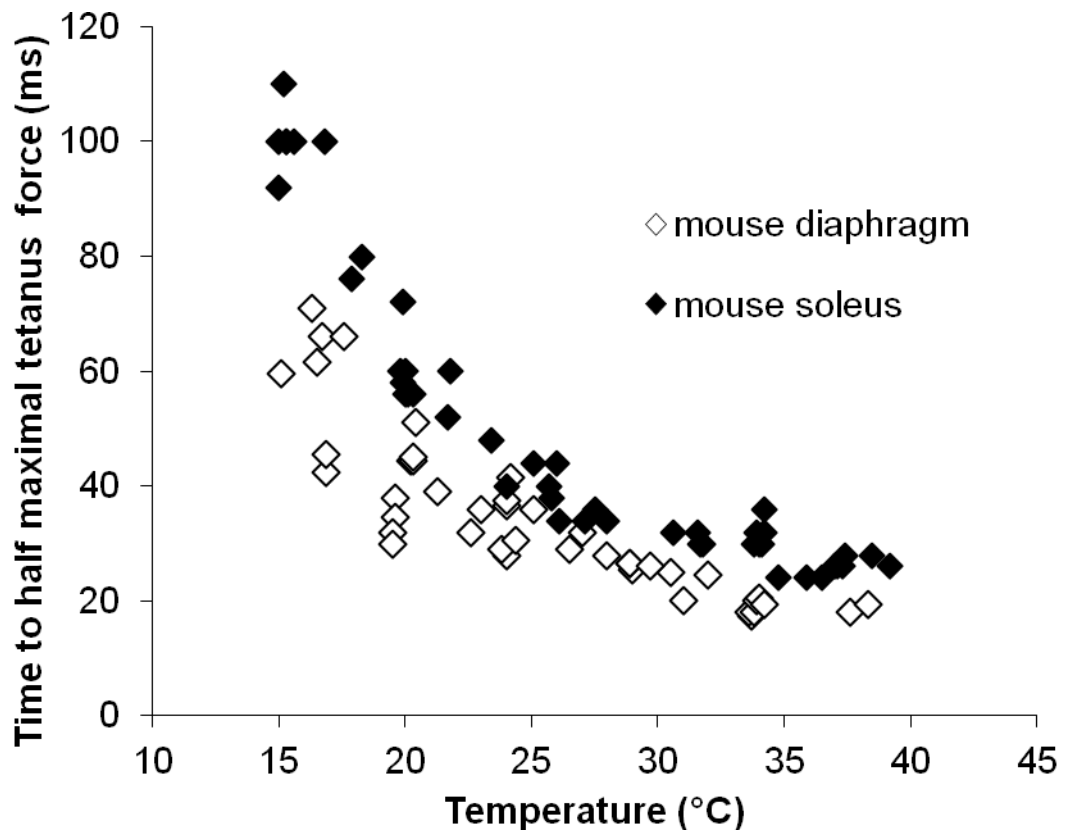
506 **Table 3** A General Linear Model of the effects of muscle mass, muscle type (i.e.  
507 diaphragm versus soleus), temperature, and time on absolute power production.

Effect	df	<i>F</i>	<i>P</i>
Intercept	1,44	0.04	0.838
Muscle mass	1,44	7.74	0.0079
Muscle type	1,44	8.33	0.0060
Temperature	1,44	1.25	0.269
Time	3,44	4.54	0.0074
Muscle mass x Temperature	1,44	43.0	<0.0001
Muscle type x Temperature	1,44	27.4	<0.0001
Temperature x Time	3,44	4.71	0.0061

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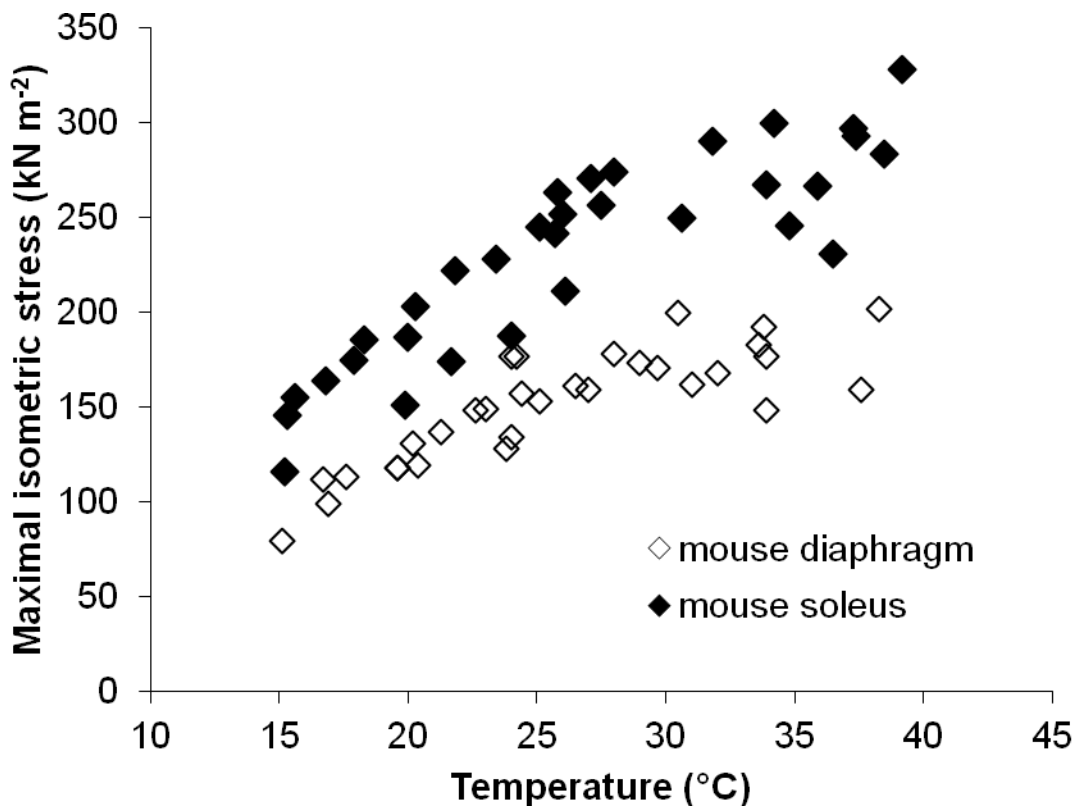
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512 **Fig. 1**

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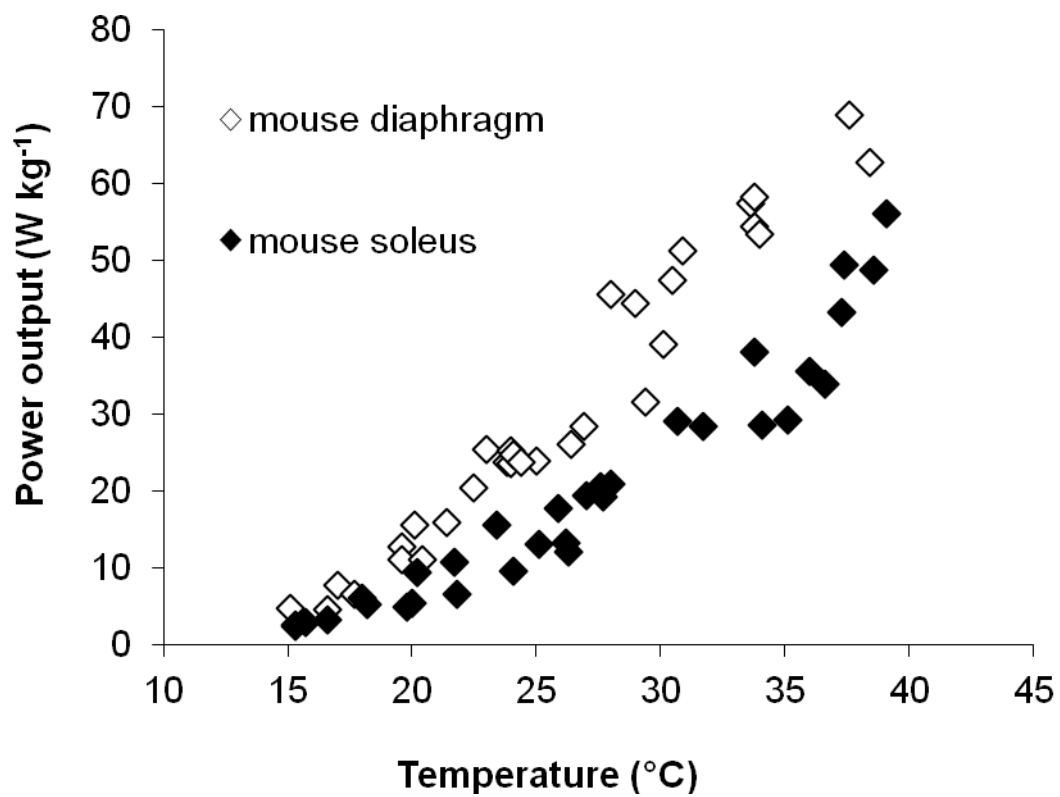




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518 **Fig. 3**

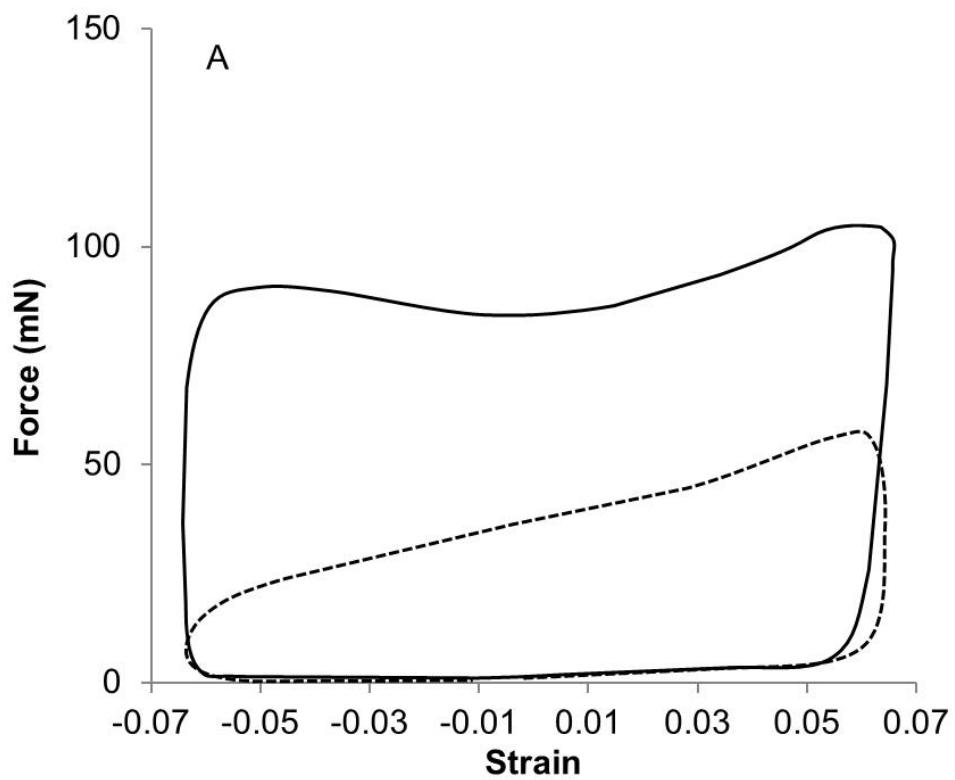
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521 **Fig. 4**

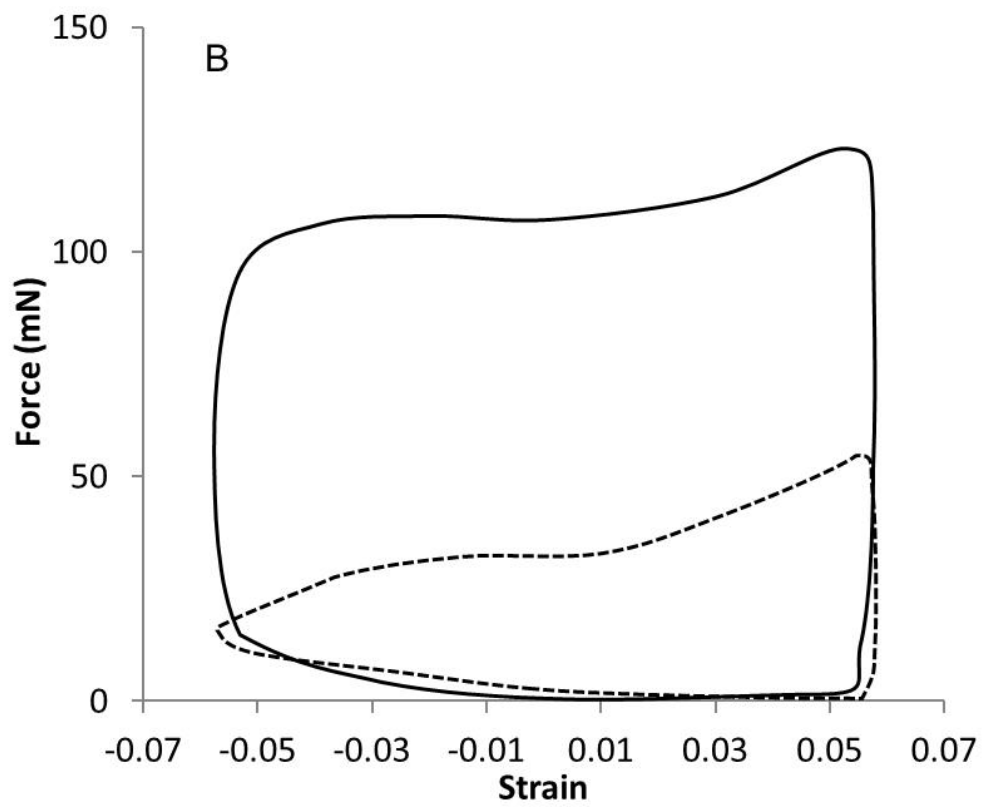
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524 **Fig. 5a**

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527 **Fig. 5b**

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