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

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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*)**

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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.

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38 **Introduction**

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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.

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98 **Materials and methods**

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101 Tissue samples

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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±0.9 g mean ± 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_2; 5% CO_2) Krebs-Henseleit solution (composition, values in mM: NaCl 118; KCl

110 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

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

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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_2; 5% CO_2) 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.

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142

143   Work-loop analysis

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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 Kreb'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   Kreb'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 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 (kN m^{-2}). Normalised muscle power output at each test temperature was calculated as

199   power output divided by wet muscle mass (W kg^{-1}).

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202   Statistical modelling

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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**

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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**

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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 it's 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.

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362 **References**

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364 Altringham JD, Block BA (1997) Why do tuna maintain elevated slow muscle
365   temperatures? Power output of muscle isolated from endothermic and ectothermic
366   fish. J Exp Biol 200:2617-2627

367 Altringham JD, Young IA (1991) Power output and the frequency of oscillatory work in
368   mammalian diaphragm muscle: the effects of animal size. J Exp Biol 157:318-389

369 Angilletta MJ Jr (2009) Thermal adaptation. A theoretical and empirical synthesis.
370   Oxford University Press, Oxford

371 Angilletta MJ Jr, Cooper BS, Schuler MS, Boyles JG (2010) The evolution of thermal
372   physiology in endotherms. Front Biosci E2:861-881

373 Bennett AF (1984) Thermal dependence of muscle function. Am J Physiol 247:R217-
374   R229

375 Bernal D, Smith D, Lopez G, Weitz D, Grimminger T, Dickson K, Graham JB (2003)
376   Comparative studies of high performance swimming in sharks II. Metabolic
377   biochemistry of locomotor and myocardial muscle in endothermic and ectothermic
378   sharks. J Exp Biol 206: 2845-2857

379 Bernal D, Donley JM, Shadwick RE, Syme DA (2005) Mammal-like muscles power
380   swimming in a cold-water shark. Nature 437:1349-1352

381 Caiozzo VJ (2002) Plasticity of skeletal muscle phenotype: Mechanical consequences.
382   Muscle Nerve 26:740-768

383 Castle PC, Macdonald AL, Philp A, Webborn A, Watt PW, Maxwell NS (2006)
384   Precooling leg muscle improves intermittent sprint exercise performance in hot, humid
385   conditions. J Appl Physiol 100:1377-1384

386 Choi IH, Cho Y, Oh YK, Jung NP, Shin HC (1998) Behavior and muscle performance in
387   heterothermic bats. Physiol Zool 71:257-266

388 Crawley MJ (2007) The R Book. Wiley, New York

389 Curtin NA, Woledge RC (1993a) Efficiency of energy conversion during sinusoidal
390   movement of white muscle fibres from the dogfish *Scyliorhinus canicula*. J Exp Biol
391   183: 137-147

392 Curtin NA, Woledge RC (1993b) Efficiency of energy conversion during sinusoidal
393   movement of red muscle fibres from the dogfish *Scyliorhinus canicula.* J Exp Biol
394   185: 195-206

395 De Ruiter CJ, Jones DA, Sargeant AJ, De Haan A (1999) Temperature effect on the rates
396   of isometric force development and relaxation in the fresh and fatigued human
397   adductor pollicis muscle. Exp Physiol 84:1137-1150

398 Donley JM, Shadwick RE, Sepulveda CA, Syme DA (2007) Thermal dependence of
399   contractile properties of the aerobic locomotor muscle in the leopard shark and
400   shortfin mako shark. J Exp Biol 210:1194-1203

401 Donley JM, Sepulveda CA, Aalbers SA, McGillivray DG, Syme DA, Bernal D (2012)
402   Effects of temperature on power output and contraction kinetics in the locomotor
403   muscle of the regionally endothermic common thresher shark (*Alopias vulpinus*). Fish
404   Physiol Biochem 38:1507-1519

405 Ducharme MB, VanHelder WP, Radomski MW (1991) Tissue temperature profile in the
406   human forearm during thermal stress at thermal stability. J Appl Physiol 71:1973-1978

407 Herrel A, James RS, Van Damme R (2007) Fight versus flight: physiological basis for
408   temperature dependent behavioral shifts in lizards. J Exp Biol 210:1762-1767

409 James RS, Altringham JD, Goldspink DF (1995) The mechanical properties of fast and
410   slow skeletal muscles of the mouse in relation to their locomotory function. J Exp Biol
411   198:491-502

412 James RS, Young IS, Cox VM, Goldspink DF, Altringham JD (1996) Isometric and
413   isotonic muscle properties as determinants of work loop power output. Pflug Archiv
414   Eur J Physiol 432:767-774

415 James RS, Tallis J, Herrel A, Bonneaud C (2012) Warmer is better: Thermal sensitivity
416   of both maximal and sustained power output in the iliotibialis muscle isolated from
417   adult *Xenopus tropicalis*. J Exp Biol 215:552-558

418 James RS (2013) A review of the thermal sensitivity of the mechanics of vertebrate
419   skeletal muscle. J Comp Physiol 183:723-733

420 Josephson RK (1985) Mechanical power output from striated muscle during cyclic
421   contraction. J Exp Biol 114:493-512

422 Kenny GP, Reardon FD, Zaleski W, Reardon ML, Haman F, Ducharme MB (2003)

423   Muscle temperature transients before, during, and after exercise measured using an
424   intramuscular multisensor probe. J Appl Physiol 94:2350-2357

425   Lovegrove BG, Heldmaier G, Ruf T (1991) Perspectives of endothermy revisited: the
426   endothermic temperature range. J Therm Biol 16:185-197

427   Marsh RL (1994) Jumping ability of anurans. In: Jones JH (ed) Comparative vertebrate
428   exercise physiology. Academic Press, San Diego, pp 51-111

429   Méndez J, Keys A (1960) Density and composition of mammalian muscle. Metabolism
430   9:184-188

431   Pinheiro J, Bates D, DebRoy S, Sarkar D, the R Development Core Team (2011) nlme:
432   linear and nonlinear mixed effects models. R PackageVersion 3.1-102

433   Rall JA, Woledge RC (1990) Influence of temperature on mechanics and energetics of
434   muscle contraction. Am J Physiol 259:R197-R203

435   Ranatunga KW (1982) Temperature-dependence of shortening velocity and rate of
436   isometric tension development in rat skeletal muscle. J Physiol 329:465-483

437   Ranatunga KW (1998) Temperature dependence of mechanical power output in
438   mammalian (rat) skeletal muscle. Exp Physiol 83:371-376

439   Refinetti R (1999) Amplitude of the daily rhythm of body temperature in eleven
440   mammalian species. J Therm Biol 2:477-481

441   Rome LC, Swank D (1992) The influence of temperature on power output of scup red
442   muscle during cyclical length changes. J Exp Biol 171:261-281

443   Saltin B, Gagge AP, Stolwijk JA (1968) Muscle temperature during submaximal exercise
444   in man. J Appl Physiol 25:679-88

445   Seebacher F, James RS (2008) Plasticity of muscle function in a thermoregulating
446   ectotherm (*Crocodylus porosus*): biomechanics and metabolism. Am J Physiol
447   294:R1024-R1032

448   Seebacher F, Tallis JA, James RS (2014) The cost of muscle power production: muscle
449   oxygen consumption per unit work increases at low temperatures in *Xenopus laevis*
450   Daudin. J Exp Biol 217: 1940-1945

451   Steury TD, Murray DL (2005) Regression versus ANOVA. Front Ecol Environ 3:356-
452   357

453   Swoap SJ, Johnson TP, Josephson RK, Bennett AF (1993) Temperature, muscle power

454   output, and limitations on burst locomotor performance of the lizard *Dipsosaurus*

455   *dorsalis*. J Exp Biol 174:185-197

456   Syme DA (2006) Functional Properties of Skeletal Muscle. In: Shadwick RE, Lauder GV

457   (ed) Randall DJ, Farrell AP (series ed.) Fish Physiology: Fish Biomechanics, vol 23,

458   Academic Press, Waltham, pp 179-240

459   Therneau T, Lumley T (2009) Survival: survival analysis, including penalised likelihood.

460   R package version 2.35-8. http://CRAN.R-project.org/package=survival

461   Wood SN (2004) Stable and efficient multiple smoothing parameter estimation for

462   generalized additive models. J Am Stat Assoc 99:673-686

463   Wooden KM, Walsberg GE (2004) Body temperature and locomotor capacity in a

464   heterothermic rodent. J Exp Biol 207:41-46

465   Yaicharoen P, Wallman K, Morton A, Bishop D (2012) The effect of warm-up on

466   intermittent sprint performance and selected thermoregulatory parameters. J Sci Med

467   Sport 15:451-456

468   Zuur AF, Leno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed Effects Models

469   and Extensions in Ecology with R. Springer, New York

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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.

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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*
T to half of maximal tetanus force:				
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
T to half tetanus relaxation :				
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	F	P
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	F	P
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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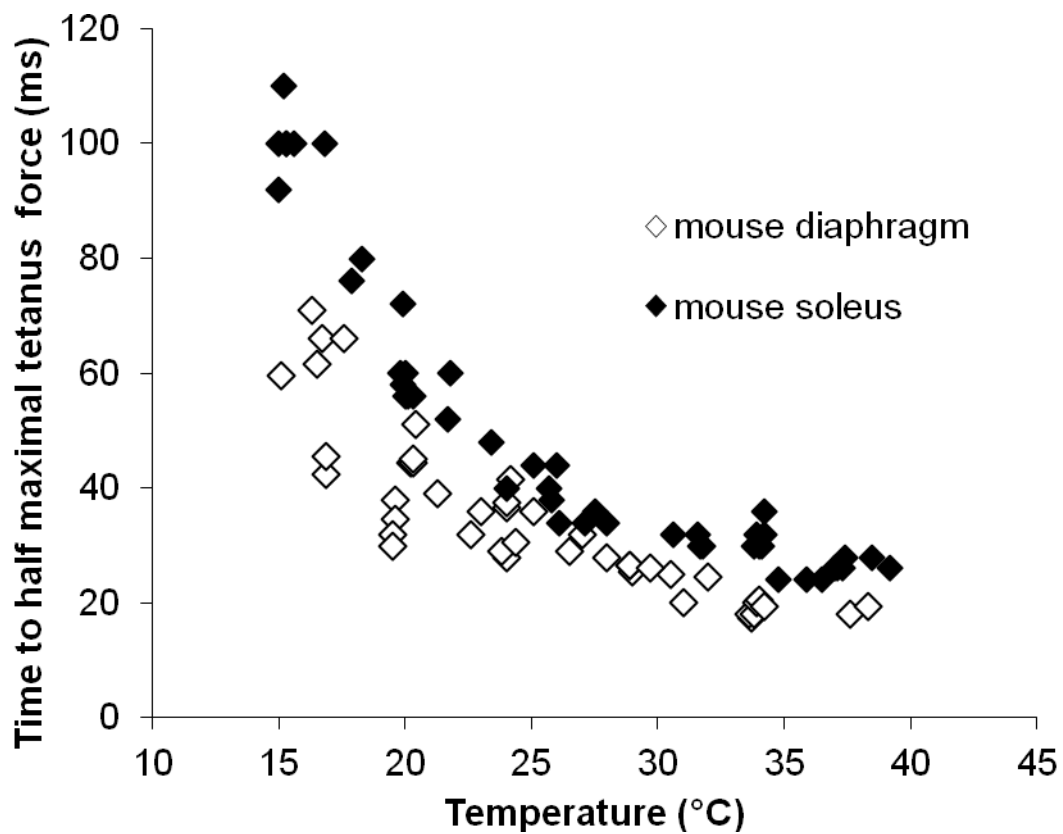
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512 **Fig. 1**

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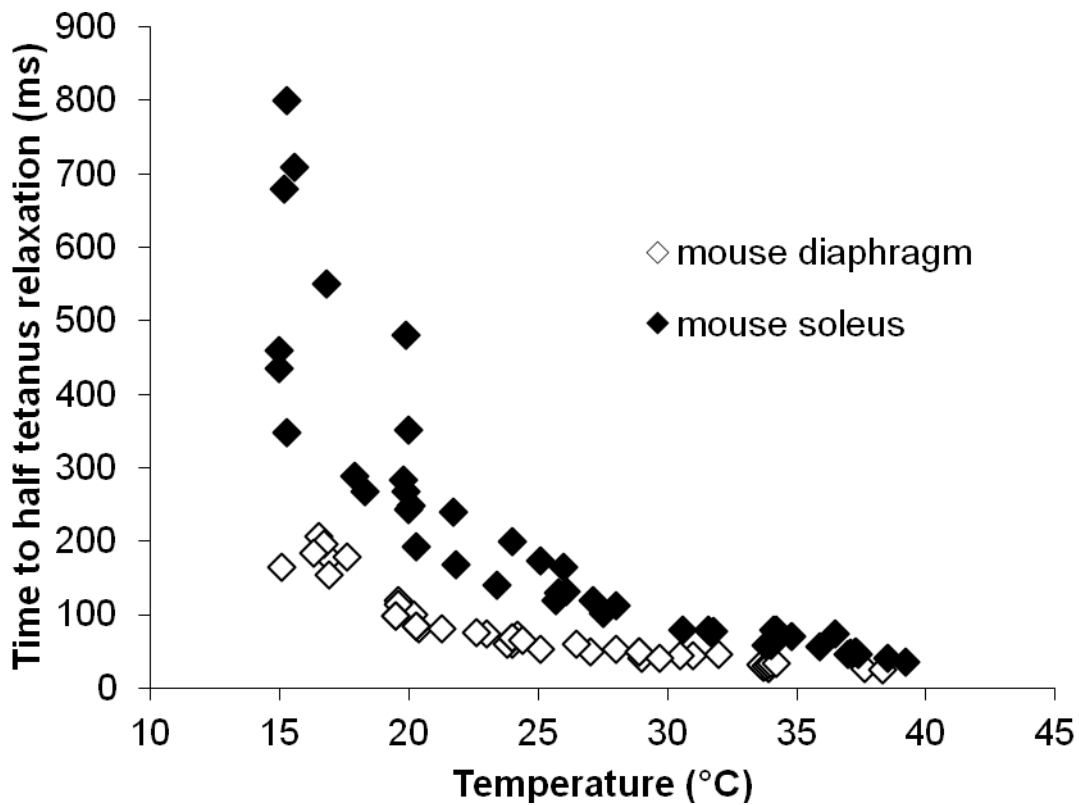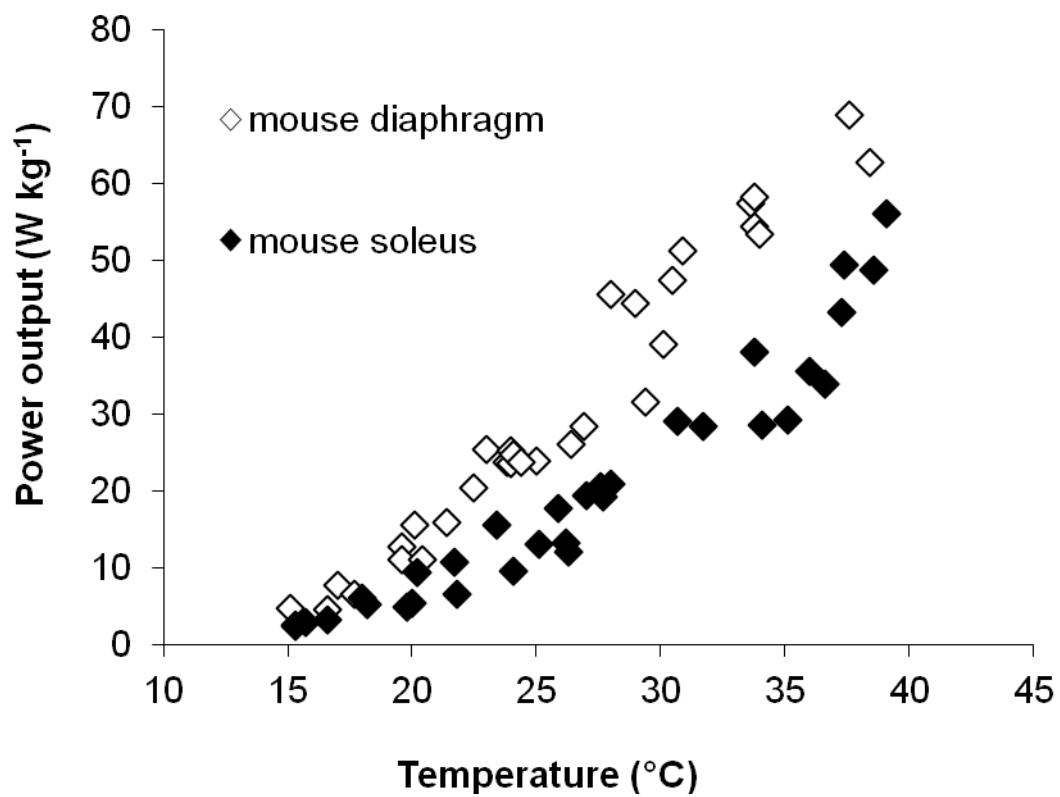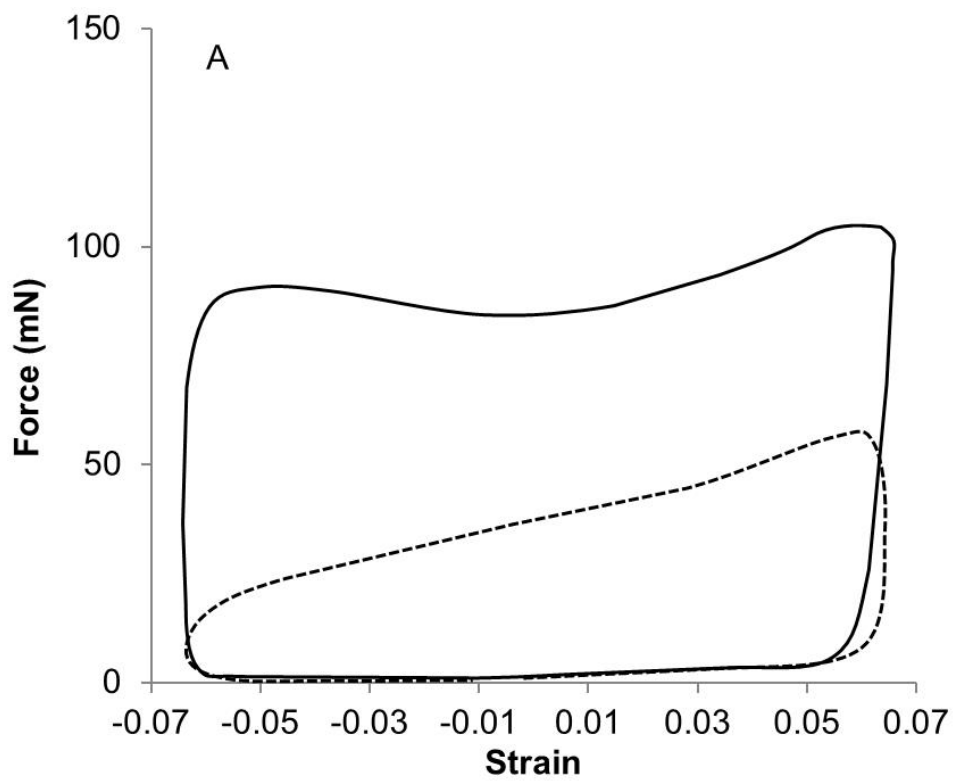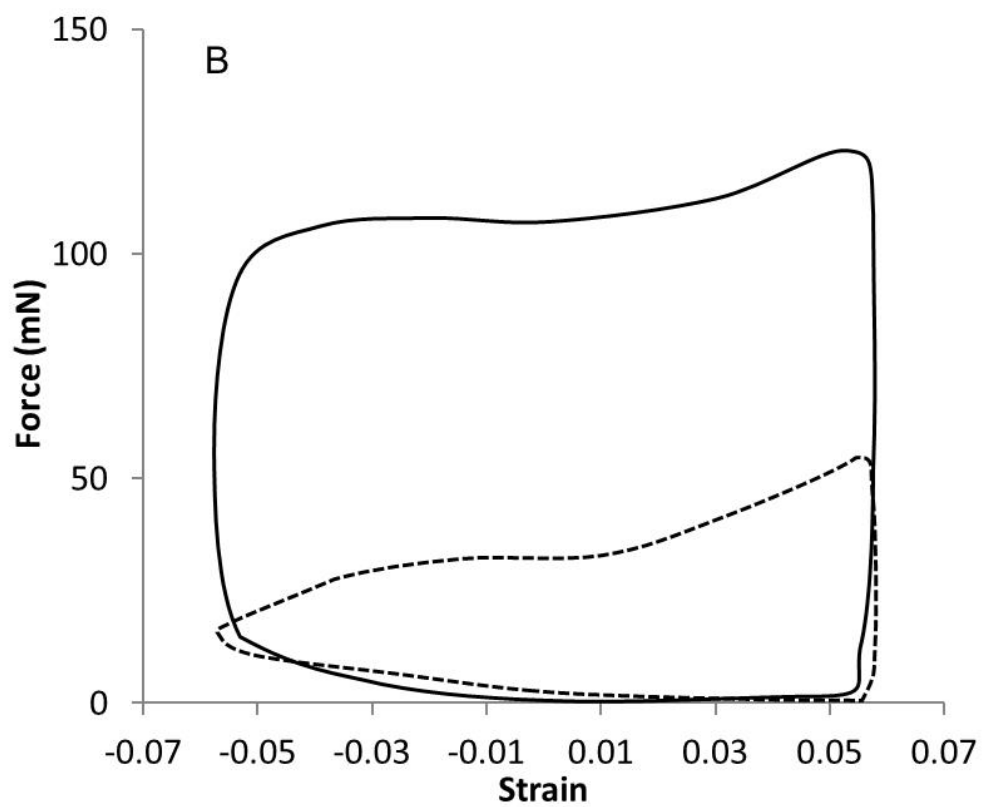
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515 **Fig. 2**

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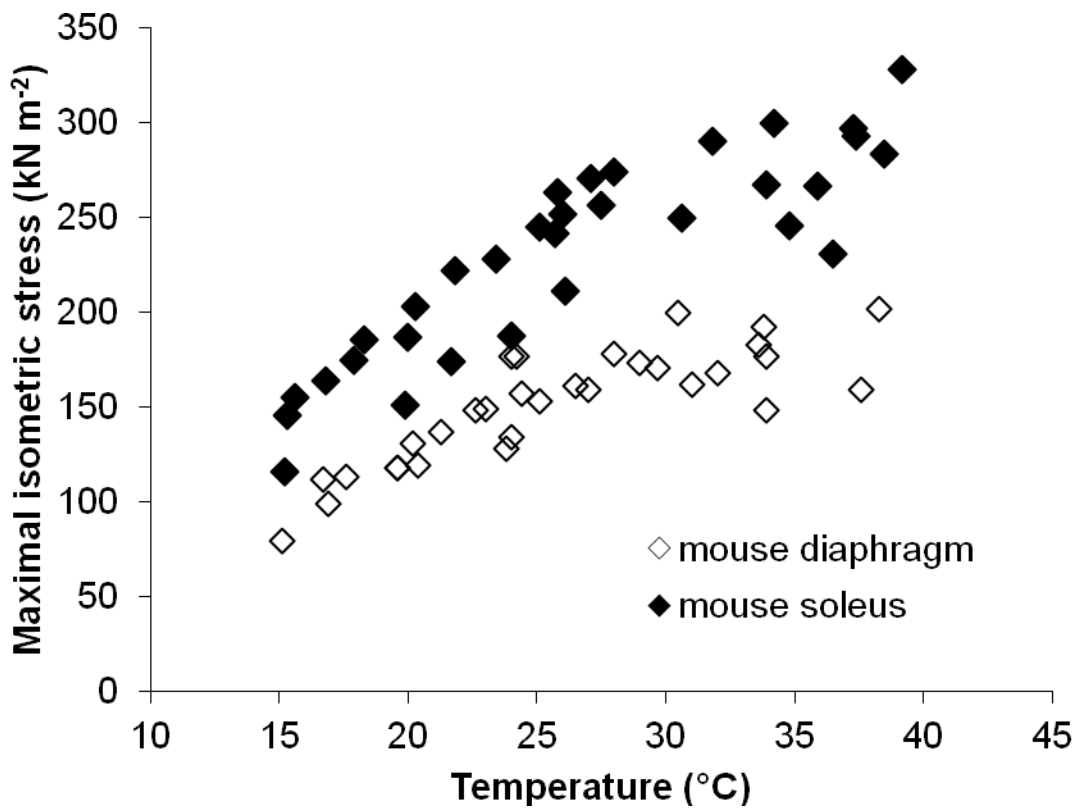
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518 **Fig. 3**

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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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