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RESEARCH ARTICLE

Daily torpor reduces mass and changes stress and power output of soleus and EDL muscles in the Djungarian hamster, *Phodopus sungorus*

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SUMMARY

Djungarian hamsters (*Phodopus sungorus*) undergo bouts of daily torpor in response to reduced photoperiod. Metabolic rate, body temperature and energy cost are reduced during torpor. The present study exposed Djungarian hamsters to two different photoperiod regimes at a room temperature of 19–21°C: long photoperiod (control, 16 h:8 h light:dark, *N*=8) and short photoperiod (torpor, 8 h:16 h light:dark, *N*=8). After 14 weeks, muscle mechanics were analyzed in each group, examining both extensor digitorum longus (EDL) muscle and soleus muscle from each individual. Control hamsters had significantly greater body mass (43%), EDL mass (24%), EDL length (9%) and soleus mass (48%) than the torpor hamsters. However, there were no significant differences between control and torpor groups in forearm length or soleus muscle length. There were no significant differences in either muscle between control and torpor hamsters in maximum twitch stress (force per unit area), tetanus force generation or relaxation times. Maximum soleus tetanic stress was 43% greater (*P*=0.039) and soleus work loop power output (*P*<0.001) was higher in torpor than in control hamsters. Maximum EDL tetanic stress was 26% greater in control than in torpor hamsters (*P*=0.046), but there was no significant effect on EDL power output (*P*=0.38). Rate of fatigue was not affected by torpor in either soleus or EDL muscles (*P*>0.43). Overall, extended use of daily torpor had no effect on the rate at which stress or work was produced in soleus and EDL muscles in Djungarian hamsters; however, torpor did increase the stress and power produced by the soleus.

Key words: atrophy, fatigue resistance, torpor, mechanics, stress.

INTRODUCTION

Many small mammals use daily torpor and/or hibernation to deal with the stresses associated with winter, such as seasonally cold temperatures and scarcity of food. For many species these seasonal responses are obligatory and are regulated *via* melatonin signals, cued by changes in photoperiod. The Djungarian hamster, *Phodopus sungorus*, is one such species whose physiology is closely regulated by photoperiod (day length), with short photoperiod exposures triggering a highly conserved suite of adjustments that includes: decreased body mass (including reductions in both fat and lean masses); decreased basal metabolic rate; reduced food intake; entry into daily torpor; and change in fur colour and density (Teubner and Bartness, 2009; Braulke et al., 2010; Warner et al., 2010). The extended use of torpor under short photoperiod (winter) conditions as well as reduced lean mass of skeletal muscles led us to wonder whether skeletal muscle performance might be altered in the short photoperiod (winter) phenotype.

Skeletal muscle is a highly adaptable tissue that responds rapidly to changes in usage and environment (Harridge, 2007; Canepari et al., 2010). Prolonged periods of muscle disuse in non-hibernating mammals are known to result in large and rapid reductions in skeletal muscle size and mechanical performance (Musacchia et al., 1988; Clark, 2009). For example, only 14 days of limb immobilisation in humans caused a 22% reduction in the force produced during isometric knee extension (Oates et al., 2010). However, studies with hibernating species that undergo cycles of multi-day torpor bouts show comparatively lower rates of change in the size and mechanical properties of skeletal muscle. More than 3 months of hibernation in black bears caused a relatively small (15%) decrease in skeletal muscle protein concentration (Tinker et al., 1998), a 29% loss of absolute isometric force in tibialis anterior muscle, and a significant reduction in fatigue resistance, but no significant changes in twitch force generation times or relaxation times (Lohuis et al., 2007b). Hibernation in golden hamsters and prairie dogs also had limited effects on isometric muscle performance (Vyskočil and Gutmann, 1977; Cotton and Harlow, 2010). Maintenance of skeletal muscle performance during periods of natural dormancy is desirable to enable animals to undertake fitness-related activities, such as those behaviours related to prey capture, predator avoidance or reproduction, as soon as they arouse from torpor.

The few previous studies of the effects of hibernation on the mechanical performance of mammalian muscle have analysed skeletal muscle when held at constant length (isometric studies). However, during natural locomotion, muscle length usually changes in a cyclic manner. The mechanical properties of muscle at constant length do not predict the performance of muscle under cyclic length changes (James et al., 1996). In contrast, the work loop technique has been used to impose cyclic length changes on muscle to simulate *in vivo* function (Josephson, 1993). For example, sartorius muscle isolated from the frog *Rana temporaria* has been assessed *via* the

work loop technique to determine the mechanical effects of 3 to 4 months of cold-water submerged hibernation (West et al., 2006).

In this study we used Djungarian hamsters to analyze the effects of prolonged exposure to short photoperiod conditions (which lead to extended use of daily torpor) (Heldmaier et al., 1999) to determine whether: (1) prolonged use of daily torpor affects the mechanical performance of skeletal muscle during maximal (sprint) and sustained (endurance) type activities as assessed using the work loop technique; and (2) responses to daily torpor differ between relatively fast twitch (extensor digitorum longus) and relatively slow twitch (soleus) skeletal muscles. This study represents the first such application of the work loop technique to a mammalian species that uses torpor/hibernation. Based upon previous studies regarding the effects of natural models of muscle disuse, we hypothesised that extended use of torpor in a small mammal such as the Djungarian hamster could result in significant skeletal muscle atrophy, but might not cause large changes in the mechanical properties of skeletal muscle.

MATERIALS AND METHODS

Animals

Adult Djungarian hamsters, *Phodopus sungorus* (Pallas 1773), were purchased from Simon's Rodents (St Neots, Cambridgeshire, UK). Hamsters of similar age and size were split into two different photoperiod regimes that are commonly used for this species (Heldmaier et al., 1999; Atgié et al., 2009; Braulke et al., 2010): control (summer; N=8), 16 h light (05:00–21:00 h), 8 h dark; and torpor (winter; N=8), 8 h light (11:00–19:00 h), 16 h dark. Room temperature was maintained between 19 and 21°C and animals were without access to a running wheel. Animals were allowed *ad libitum* access to water and food (expanded rodent diet, Special Diet Services, Witham, Essex, UK). Those kept under short photoperiod conditions soon changed to a predominantly white winter pelage and entered daily torpor. After 14 weeks, in constant summer or winter photoperiod, animals were euthanised and muscle samples were taken for mechanical performance measurements. Individuals from the daily torpor group were only euthanised when they were in torpor.

Dissection

Hamsters were euthanised by dislocation of the neck in accordance with the British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1. Hamster body mass was determined to the nearest 0.01 g using an electronic balance. Both hindlimbs were removed and were simultaneously used for dissection. Soleus muscle was isolated from the right hindlimb and extensor digitorum longus (EDL) was isolated from the left hindlimb at room temperature (19–21°C) in oxygenated (95% O_2; 5% CO_2) Krebs–Henseleit solution (composition, values in mmol l^{-1}: NaCl 118; KCl 4.75; $MgSO_4$ 1.18; $NaHCO_3$ 24.8; KH_2PO_4 1.18; glucose 10; $CaCl_2$ 2.54; pH 7.55 at room temperature prior to oxygenation). EDL and soleus were chosen as representative limb skeletal muscles of predominantly fast fibre and slow fibre types, respectively (Mattson et al., 2002). A small piece of bone was left attached at the end of both the proximal and distal tendons of each soleus muscle preparation. Aluminium foil clips were wrapped around the tendons at either end of each soleus muscle. A length of bone was left attached to the proximal end of each EDL muscle preparation. An aluminium foil clip was wrapped around the distal tendons of each EDL muscle.

Isometric studies

One soleus muscle and one EDL muscle from each hamster were analysed simultaneously on different sets of equipment. The bone or foil clip at either end of each muscle preparation was clamped *via* crocodile clips to a strain gauge (UF1, Pioden Controls Ltd, Canterbury, Kent, UK) at one end and a motor arm (V201, Ling Dynamics Systems, Royston, Hertfordshire, UK) attached to a linear variable displacement transformer (DFG 5.0, Solartron Metrology, Bognor Regis, Sussex, UK) at the other. Each muscle was maintained at 37.0±0.5°C in circulating oxygenated Krebs solution. Each preparation was stimulated *via* parallel platinum electrodes whilst held at constant length to generate a series of twitches. Stimulus amplitude and muscle length were adjusted to maximise isometric twitch force. Stimulus pulse width was set at 1.5 ms. The muscle length that yielded maximal twitch force was measured to the nearest 0.1 mm using a dissecting microscope fitted with an eyepiece graticule. An isometric tetanic force response was elicited by subjecting the soleus muscle to a 350 ms train of stimulation at a frequency of 140 Hz and the EDL to a 250 ms train of stimulation at a frequency of 200 Hz. Stimulation frequency was then altered to determine maximal tetanic force. Time to half peak tetanic force and time from last stimulus to half tetanic force relaxation were measured. A rest period of 5 min was allowed between each tetanic response.

Work loop studies

The work loop technique was used to determine the power output of muscles during cyclical length changes (Josephson, 1993). Each muscle preparation was subjected to a set of four sinusoidal length changes, starting from the length that was optimal for maximal twitch force production. The muscle stimulation parameters found to yield maximal isometric force were used (stimulation frequency and amplitude). Electrical stimulation and length changes were controlled *via* a D/A board (KUSB3116, Keithley Instruments, OH, USA) and a customised program produced using Testpoint software (CEC Testpoint version 7, Measurement Computing, Norton, MA, USA). For each work loop cycle, muscle force was plotted against muscle length to generate a work loop, the area of which equated to the net work produced by the muscle during the cycle of length change (Josephson, 1993). The net work produced was multiplied by frequency of length change cycles to calculate net power output. The total strain of length change cycles was maintained at 0.10 throughout all experiments (i.e. ±5% of resting muscle length). The cycle frequency of length change was altered, up and down within the range of 1 to 10 Hz for soleus and between 2 and 15 Hz for EDL, to generate power output *versus* cycle frequency curves. During these length changes, the muscle was subjected to phasic stimulation (active work loop cycles). Every 5 min the muscle was subjected to a further set of four work loop cycles with stimulation duration and stimulation phase parameters being altered until maximum net work was achieved at each cycle frequency. Before the fatigue run, a set of control sinusoidal length change and stimulation parameters were imposed on the muscle every three to four sets of work loops to monitor variation in the muscle's ability to produce power and/or force. Any variation in power was found to be due to a matching change in ability to produce force. Therefore, the power produced by each preparation, prior to the fatigue run, was corrected to the control run that yielded the highest power output, assuming that alterations in power generating ability were linear over time. All muscles still produced over 80% of maximal control run power output by the end of each experiment. Prior to the fatigue run, most muscle preparations varied by 5 to 10% in control run power over the time course of the experiment. On completion of the power-output–cycle-frequency curve, each muscle was then subjected to a fatigue run consisting of 100 work loop cycles at a cycle frequency of 3 Hz at a stimulation frequency of half that found to generate

maximal force, i.e. during the fatigue run the muscles were stimulated to generate submaximal power.

At the end of the muscle mechanics experiments, the bones and tendons were removed and each muscle was blotted on absorbent paper to remove excess Krebs solution. Wet muscle mass was determined to the nearest 0.01 mg using an electronic balance. Mean muscle cross-sectional area was calculated from muscle length and mass assuming a density of 1060 kg m^{-3} (Méndez and Keys, 1960). Maximum isometric muscle stress (force per unit area) was then calculated as maximum tetanic force divided by mean cross-sectional area (kN m^{-2}). Normalized muscle power output was calculated in two ways: as power output divided by wet muscle mass (W kg^{-1} muscle mass) and as power output divided by body mass (W kg^{-1} body mass).

To quantify animal size independently of body mass, the distance from the elbow to the wrist in the forelimb was measured using digital Vernier callipers and was referred to as forearm length.

Statistical analysis

Any dispersion measurements are given as standard error. Significance was taken at the level of $P<0.05$. In most cases, control and torpor results were compared using independent sample *t*-tests, with correction for unequal variances where appropriate. Comparison of control and torpor power-output–cycle-frequency data was performed using two-factor ANOVA, with experimental treatment and cycle frequency as the factors. This approach allowed the interaction term to indicate whether torpor affected the shape of the power-output–cycle-frequency curve. If one of the experimental muscle groups had faster mechanical properties, there should be a rightward and upward shift in the power-output–cycle-frequency curve (James et al., 1995). A two-factor repeated-measures ANOVA was used to analyse the effects of torpor on fatigue resistance with experimental group (between-subjects factor) and loop number (essentially the time elapsed during the fatigue run; within-subjects factor) as the two factors. Mauchly's test for sphericity indicated that there were significant differences in the variance of differences between the torpor and control groups. Therefore, the Greenhouse–Geisser correction was used to correct the within-subject *P*-values from the repeated-measures ANOVA. Independent sample Student's *t*-tests were used as *post hoc* tests for differences between torpor and control muscle performance at each cycle frequency in Fig. 1, when ANOVA demonstrated a significant difference in power output produced in power-output–cycle-frequency curves.

The truncated product method (Zaykin et al., 2002) was used to combine all the *P*-values in this study to determine whether there was a bias from multiple hypothesis testing. The truncated product method *P*-value was <0.001, indicating that the results are not biased by multiple comparisons.

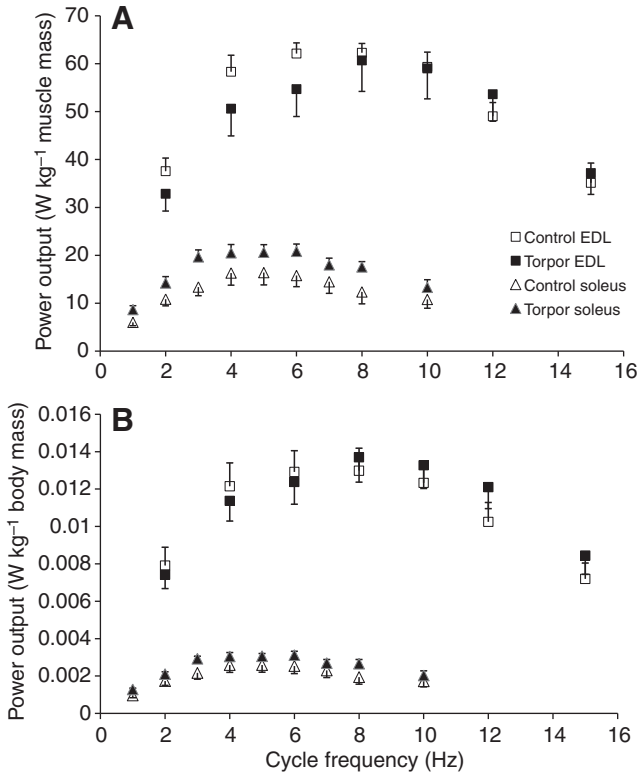

Fig. 1. Effects of torpor on the power output generated by Djungarian hamster extensor digitorum longus (EDL) and soleus muscles (represented by squares and triangles, respectively). Control and torpor groups are represented by open and closed symbols, respectively. (A) Power output normalised to muscle mass; (B) power output normalised to body mass. Soleus work loop power output was higher in torpor than in control hamsters ($P<0.001$). There was no effect of hibernation on EDL power output ($P>0.37$). Data are means ± s.e.m., $N=8$.

RESULTS

Morphology

The control group of hamsters had 43% greater body mass ($t=3.38$, $P=0.005$; Table 1), 24% greater EDL mass ($t=2.61$, $P=0.02$), 9% greater EDL length ($t=3.13$, $P=0.007$) and 48% greater soleus mass ($t=2.80$, $P=0.014$) than the torpor group. However, there were no significant differences between control and torpor groups in terms of forearm length or soleus muscle length (Table 1).

Isometric properties

There were no significant differences between control and torpor hamster groups in tetanus force generation or tetanus force relaxation

Table 1. Effects of torpor on Djungarian hamster morphology

	EDL			Soleus		
	Control	Torpor	*P*	Control	Torpor	*P*
Muscle length (mm)	6.6±0.1	6.1±0.1	0.007	4.8±0.4	5.3±0.2	0.27
Muscle mass (mg)	6.22±0.31	4.99±0.35	0.02	4.86±0.53	3.28±0.20	0.014
Body mass (g)	31.2±2.4	21.9±1.4	0.005			
Forearm length (mm)	20.1±0.7	18.7±0.1	0.06			

Data are means ± s.e.m. *P*-values are given for independent sample Student's *t*-tests.
EDL, extensor digitorum longus.

Table 2. Effects of torpor on skeletal muscle isometric tetanus kinetics and maximal stress in the Djungarian hamster

	EDL			Soleus		
	Control	Torpor	*P*	Control	Torpor	*P*
Time to half peak tetanus (ms)	16.3±0.9	15.4±0.8	0.47	32.5±2.4	33.0±3.0	0.89
Time from last stimulus to half tetanus relaxation (ms)	23.9±1.0	22.8±0.9	0.45	38.7±2.3	39.6±4.5	0.87
Maximum twitch stress (kN m^{-2})	56.5±3.2	44.9±7.4	0.17	14.1± 2.5	19.1±2.4	0.17
Maximum tetanus stress (kN m^{-2})	270±12	215±22	0.046	74.4±7.3	106±12	0.039

Data are means ± s.e.m. *P*-values are given for independent sample *t*-tests.

times in EDL or soleus muscles (t<0.79, P>0.44 in each case; Table 2). Maximum twitch stress (force per unit area) was not significantly different between control and torpor hamster groups in either EDL or soleus muscles (t<1.46, P=0.17 in both cases; Table 2). Maximum tetanic stress of EDL muscle was 26% greater in control hamsters than in torpor hamsters (t=2.19, P=0.046; Table 2). Maximum tetanic stress of soleus muscle was 43% greater in torpor hamsters than in control hamsters (t=2.28, P=0.039; Table 2).

Work loop performance

There was no significant effect of torpor on power output produced by EDL when normalised to either muscle mass (F=0.79, P=0.38; Fig. 1A) or body mass (F=0.50, P=0.48; Fig. 1B). Cycle frequency had a significant effect on power output of hamster EDL muscle (F>9.1, P<0.001 in each case; Fig. 1), but there was no significant interaction between cycle frequency and torpor (F<0.51, P>0.80 in each case), suggesting that torpor had no effect on the shape of the power-output–cycle-frequency curve. These findings indicate that the intrinsic contractile rate and power output of EDL muscle was maintained during torpor relative to both muscle mass and body mass.

The soleus muscle from torpor hamsters produced significantly higher power output compared with that from control hamsters when normalised to either muscle mass (F=23.9, P<0.001; Fig. 1A) or body mass (F=15.0, P<0.001; Fig. 1B). *Post hoc* analysis determined that power output was significantly greater at 1 Hz cycle frequency in both cases (t>2.25, P<0.041), along with 3 Hz cycle frequency when normalised to muscle mass (t=2.73, P=0.016). Cycle frequency caused a significant effect on power output in soleus muscles (F>8.76, P<0.001 in each case), but there was no interaction between the effects of cycle frequency and torpor (F<0.24, P>0.98 in each case). These results suggest that torpor caused an increase in the power produced by soleus muscle of hamsters (regardless of whether power was normalised to muscle mass or body mass), but that this was achieved *via* an increase in stress (force per unit area), without a change in contractile rate of the muscle as the shape of the power-output–cycle-frequency curve was unaffected.

Both EDL and soleus muscles underwent significant fatigue over the first 50 work loops of the fatigue run (F>64.8, P<0.001 in each case; Fig. 2). However, torpor had no significant effect on fatigue resistance in either muscle (F<0.65, P>0.43 in each case).

DISCUSSION

In line with our original hypotheses, successive bouts of daily torpor led to smaller skeletal muscles and limited changes in contractile rate and fatigue resistance in soleus and EDL muscles. However, daily torpor caused significant changes in the isometric stress (force per unit area) generated by soleus and EDL muscles, and a significant increase in the normalised power output (power produced per muscle mass) produced by the soleus.

Morphology

In the present study, control (long photoperiod, summer) Djungarian hamsters had 43% higher body mass than torpor (short photoperiod, winter) hamsters and similarly greater EDL (25%) and soleus (48%) muscle masses after 14 weeks in different photoperiods. A progressive reduction of 15 to 25% in Djungarian hamster body mass under short photoperiods has been widely reported over similar time periods of daily torpor (Atgié et al., 2009; Teubner and Bartness, 2009; Braulke et al., 2010; Jethwa et al., 2010), as well as reduced lean muscle mass (*ca.* 20% reduction in quadriceps mass of male hamsters) (Braulke et al., 2010). In the present study there was a small (9%) difference in EDL length between control and torpor hamsters, no significant differences in soleus muscle length, a tendency for longer (8%) forearm length and noticeably larger amounts of body fat (R.S.J., unpublished) in control hamsters. Therefore, there appears to have been significant skeletal muscle atrophy and reduction of body fat in response to torpor conditions. Some previous studies on hibernation have also found significant skeletal muscle atrophy; for example, 13 to 30% skeletal muscle mass loss in a number of species of hibernating mammals (Musacchia et al., 1989; Rourke et al., 2004; Cotton and Harlow, 2010). However, in other previous work, there was no evidence of skeletal muscle atrophy during hibernation (Rourke et al., 2004; Lohuis et al., 2007a; Lee et al., 2008; Cotton and Harlow, 2010). Muscle atrophy can be caused by reductions in the load or activity experienced by the muscle (Musacchia et al., 1988; Narici and Maganaris, 2007; Phillips et al., 2009). Such muscle disuse atrophy is usually higher in muscles of slower fibre type that would normally be active for a greater

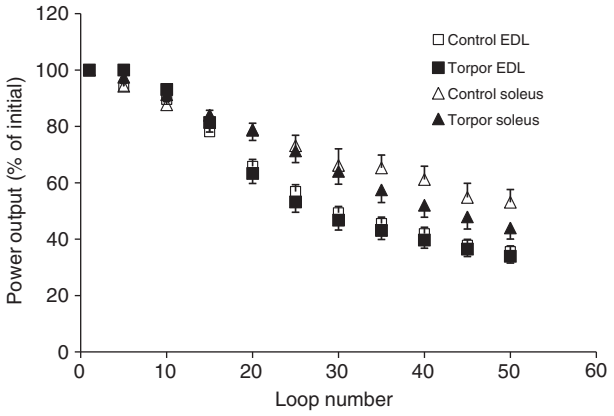

Fig. 2. Torpor did not affect skeletal muscle fatigue resistance during a series of work loops for Djungarian hamster EDL and soleus muscles (represented by squares and triangles, respectively; P>0.43 in each case). Control and torpor groups are represented by open and closed symbols, respectively. Data are means ± s.e.m., N=8 in each case.

proportion of the day. Although the hamsters in the present study would still be active on a daily basis, the use of daily torpor appears to have resulted in a large enough decrease in muscular activity and/or loading to cause a reduction in muscle mass, especially in the slower soleus muscle.

Assessment of the contractile rate of skeletal muscle

The isometric results from the present study demonstrate that after 14 weeks of short photoperiod in Djungarian hamsters there were no alterations in tetanus force generation time or tetanus force relaxation time in EDL or soleus muscles. A previous study also found no change during hibernation in the rates of isometric tetanus stress development in biceps brachii muscle from bats (Lee et al., 2008). Most previous studies have analysed isometric twitch times, finding that hibernation did not change twitch force generation times or twitch force relaxation times in a range of mammals (Vyskočil and Gutmann, 1977; Lohuis et al., 2007b; Cotton and Harlow, 2010). In contrast, twitch force generation time significantly decreased during hibernation in EDL muscle in white-tailed prairie dogs (Cotton and Harlow, 2010), whereas twitch force generation time was significantly increased during hibernation in biceps femoris muscle in captive brown bears (Hershey et al., 2008), although in both cases twitch force relaxation time did not change. Therefore, hibernation or extended use of daily torpor does not necessarily affect rates of force generation or force relaxation in skeletal muscles, suggesting that often there is no change in the intrinsic contractile rate of the muscle in mammals undergoing extended periods of hypometabolism. These findings suggest that skeletal muscle quality and function is maintained throughout torpor and hibernation regardless of changes in body mass or fat stores.

In the present study we also analysed work loop performance to assess the effect of torpor on muscle power output. Torpor did not change the shape of the work loop power-output–cycle-frequency curves, supporting the findings, from isometric studies, that there was no change in the intrinsic contractile rate of the muscles analysed. The work loop results from the present study are broadly similar (showing no or very limited change in power output) to those found in response to hibernation and aestivation in frogs (West et al., 2006; Symonds et al., 2007), but contrast with the finding that clinical and experimental models of muscle disuse in non-hibernating mammals cause a shift towards faster muscle fibre type (Musacchia et al., 1988; Canepari et al., 2010; James, 2010). Recent studies assessing the relative expression of myosin isoforms in hibernating mammals have demonstrated either a fast to slow muscle fibre type transition or no change in fibre type. There were fast to slow shifts in proportional expression of myosin heavy chain in gastrocnemius and plantaris muscle in ground squirrels (Rourke et al., 2004; Nowell et al., 2011), and soleus in white tailed prairie dogs and biceps femoris in black bears (Rourke et al., 2006). However, there were no changes in myosin heavy chain expression in the soleus or diaphragm muscle in ground squirrels (Rourke et al., 2004), pectoral muscle in bats (Lee et al., 2008), gastrocnemius in black bears (Rourke et al., 2006) or biceps femoris in captive brown bears (Hershey et al., 2008). Where significant changes in myosin heavy chain expression occur, we would expect concomitant alterations in the contractile rate of skeletal muscle (Harridge, 2007; Canepari et al., 2010). As there were no changes in contractile rate in the muscles in the present study, it seems unlikely that myosin heavy chain expression has changed.

Overall it seems that natural models of muscle disuse have lower and different disuse stimuli when compared with muscle disuse in non-hibernators. The large reductions in muscle mass seen in the present study are still relatively small when compared with those that occur during muscle disuse in non-hibernators. The reductions in muscle mass seen in the present study were not associated with the changes in intrinsic contractile rate seen in non-hibernating mammals.

Assessment of stress and power of skeletal muscle

In the present study, the EDL muscle of torpor hamsters produced 20% less isometric tetanic stress (force per unit area) than the EDL of control hamsters; however, the soleus muscle of torpor hamsters produced 43% greater tetanic stress than controls. Similar trends, of the same magnitude, although not reaching statistical significance, were found in the twitch stresses of EDL and soleus muscles. Previously, twitch stress has been shown to decrease with hibernation in EDL muscle from both white-tailed and black-tailed prairie dogs, but no change was found in soleus muscle in these two species (Cotton and Harlow, 2010). Hibernation did not cause a change in isometric tetanic stress in biceps brachii muscle in bats (Lee et al., 2008). The higher isometric stress (force per cross-sectional area) found in torpor soleus muscle in the present study was also matched by significantly greater power per unit mass of torpor muscle in the work loop studies, when compared with the soleus from control hamsters. There are no previous studies with which to compare the effects of hibernation on mammalian muscle power output. However, the findings from the present and previous studies suggest that during daily torpor and hibernation, both twitch and tetanus muscle stress are more likely to decrease in relatively fast muscles, but that twitch stress, tetanus stress and normalised power output are likely to remain the same or increase in relatively slow muscles. Possible reasons for changes in muscle stress and normalised power output, without changes in contractile rate, could include dehydration or an increase in the proportion of the muscle that is composed of contractile elements, as opposed to non-contractile components, e.g. an increased concentration of myosin. In humans and mice, vastus lateralis and soleus muscle disuse is associated with reductions in absolute muscle force and muscle stress (force per unit area), with the latter caused by a reduction in myosin concentration (Canepari et al., 2010). Previous studies on hibernation have found a significant increase in protein concentration in ground squirrel plantaris muscle (Rourke et al., 2004), significant decreases in skeletal muscle protein concentration in black-tailed prairie dogs (Cotton and Harlow, 2010) and captive brown bears (Hershey et al., 2008), but no significant changes in skeletal muscle protein concentration in other muscles of golden-mantled ground squirrels (Musacchia et al., 1989; Rourke et al., 2004), white-tailed prairie dogs (Cotton and Harlow, 2010) or wild black bears (Lohuis et al., 2007a). These previous studies showed no difference in trends between relatively fast and relatively slow skeletal muscles, but demonstrate that changes in muscle stress, such as those seen in the present study, could be due to changes in myosin concentration. In the present study, the observed decreases in absolute skeletal muscle force and power should not affect the locomotory performance of hamsters because the decreases in muscle performance are more than compensated by an overall decrease in body mass, i.e. although muscle mass, and thus absolute muscle force, have decreased, there is a smaller hamster to move on arousal. We have demonstrated that the muscle power output produced by the soleus and EDL is the same or higher in torpor animals when normalised to body mass. This suggests that hamsters may be able to maintain skeletal muscle performance ready for fitness-related activities, such as escape responses, finding food and locating a mate, upon emergence from torpor.

In the present study, several weeks of daily torpor did not affect work loop fatigue resistance in EDL or soleus muscle. Hibernation has previously been found to reduce fatigue resistance during isometric tetanus studies in the tibialis anterior muscle of wild black bears (Lohuis et al., 2007b). In contrast, 9 months of aestivation did not cause significant changes in work loop fatigue resistance of either sartorius or ileofibularis muscles in a frog (Symonds et al., 2007) and studies using experimental models of disuse in mammalian hibernators have generally found no change in skeletal muscle fatigue resistance (Musacchia et al., 1988). Therefore, it appears that torpor and hibernation do not affect fatigue resistance during muscle actions that more closely simulate locomotion, suggesting that animals are able to maintain muscle endurance and remain ready to undertake locomotor activities on arousal.

CONCLUSIONS

We have extended the available data concerning the effects of hibernation on mammalian skeletal muscle by showing that an alternative strategy of winter survival (prolonged use of daily torpor) also has similar consequences for muscle. Overall, the present and previous studies demonstrate that, despite a significant amount of skeletal muscle atrophy by hamsters and some hibernating species, the animals are able to preserve skeletal muscle mechanics in readiness for locomotory demands upon arousal. Furthermore, we provide the first data from work loop power output and fatigue resistance experiments for any mammal that uses seasonal hypometabolism (torpor or hibernation). Between torpor bouts, many mammals (including the hamsters used in the present study) arouse and undertake shivering thermogenesis and exercise, periodically elevating cellular and biochemical processes from the low levels seen in the hypometabolic state (Storey et al., 2010). Such arousal bouts could help to reduce the muscle disuse stimulus for both skeletal muscle atrophy and changes in mechanical properties, in keeping with recent findings that low volume exercise can reduce muscle disuse atrophy in humans (Oates et al., 2010). Arousal from torpor bouts in bats coincides with increases in protein synthesis *via* activation of mammalian target of rapamycin (mTOR), helping to stop or reduce muscle atrophy during hibernation (Lee et al., 2010). The amount of muscle atrophy can dramatically differ between skeletal muscles in the same species, with different molecular pathways seemingly responsible for such differences (Nowell et al., 2011). For example, decreased expression of myostatin during hibernation in ground squirrel soleus and diaphragm muscle is likely to be linked with minimising atrophy in these muscles. Relative levels of myostatin mRNA are upregulated in Djungarian hamsters under natural photoperiod conditions in winter and are negatively correlated with muscle mass, which suggests that the well-known function of myostatin as a negative regulator of muscle mass may play a prominent role in winter atrophy of skeletal muscle in this species (Braulke et al., 2010). In hibernating bats, there is significantly greater expression of heat shock protein 70, which acts as a molecular chaperone to protect cells affected by stress (Lee et al., 2008). Recent studies on experimental muscle disuse in non-hibernating animals indicate potential future directions for increasing our understanding of the mechanisms regulating muscle atrophy. Knockout studies have linked the NF-κβ transcription factor p50 subunit and the NF-κβ co-transactivator Bcl-3 with the regulation of muscle atrophy during hindlimb unloading in mice, directly affecting seven genes, including MuRF1 (TRIM 63), previously shown to be involved in muscle atrophy (Wu et al., 2011). In turn, soleus muscles in MuRF1 knockout mice have been found to show no muscle atrophy in response to hindlimb unloading (Labeit et al., 2010). Study of such factors, coupled with the assessment of muscle atrophy and mechanical performance in models of torpor and hibernation, could further our understanding of how these seasonal mammals show better maintenance of skeletal muscle size and performance than non-hibernators during periods of muscle disuse.

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