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RESEARCH ARTICLE

Skeletal muscles of hibernating brown bears are unusually resistant to effects of denervation

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SUMMARY

Hibernating bears retain most of their skeletal muscle strength despite drastically reduced weight-bearing activity. Regular neural activation of muscles is a potential mechanism by which muscle atrophy could be limited. However, both mechanical loading and neural activity are usually necessary to maintain muscle size. An alternative mechanism is that the signaling pathways related to the regulation of muscle size could be altered so that neither mechanical nor neural inputs are needed for retaining strength. More specifically, we hypothesized that muscles in hibernating bears are resistant to a severe reduction in neural activation. To test this hypothesis, we unilaterally transected the common peroneal nerve, which innervates ankle flexor muscles, in hibernating and summer-active brown bears (*Ursus arctos*). In hibernating bears, the long digital extensor (LDE) and cranial tibial (CT) musculotendon masses on the denervated side decreased after 11 weeks post-surgery by 18±11 and 25±10%, respectively, compared with those in the intact side. In contrast, decreases in musculotendon masses of summer-active bears after denervation were 61±4 and 58±5% in the LDE and CT, respectively, and significantly different from those of hibernating bears. The decrease due to denervation in summer-active bears was comparable to that occurring in other mammals. Whole-muscle cross-sectional areas (CSAs) measured from ultrasound images and myofiber CSAs measured from biopsies decreased similarly to musculotendon mass. Thus, hibernating bears alter skeletal muscle catabolic pathways regulated by neural activity, and exploration of these pathways may offer potential solutions for disuse atrophy of muscles.

Key words: atrophy, hibernation, bear.

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INTRODUCTION

Hibernating bears stay within the confined space of a den for 5 to 6 months. During mid-hibernation, bears will maintain a curled fetal-like position to conserve energy and water, and they will change position as infrequently as once every 2 days (Toien et al., 2011). Despite this prolonged inactivity, skeletal muscle atrophy is minimal in hibernating American black bears (*Ursus americanus*) and brown bears (*Ursus arctos*) (Tinker et al., 1998; Harlow et al., 2001; Lohuis et al., 2007; Hershey et al., 2008). Specifically, when comparing hibernation and non-hibernation muscle properties, there is no statistically significant change in muscle fiber size (Tinker et al., 1998; Hershey et al., 2008) and maximal force elicited by electrical stimulation decreases by only 23% (Harlow et al., 2001). This is in contrast to other mammals, for which weight-bearing activities are normally crucial to maintaining skeletal muscle mass, morphology and protein composition (Baldwin and Haddad, 2001). Decreases for maximal muscle force and size on the order of 60% are common outcomes after mechanical unloading of leg muscles (Caiozzo et al., 2007). Thus, hibernating bears are unusual among mammals in that they are able to overcome the lack of physiological stimuli that are key factors regulating muscle plasticity. However, the mechanisms responsible for this attribute have not been identified.

A mechanism that has been hypothesized is that regular neural activation of muscles could occur and help offset the effects of the mechanical unloading. Namely, shivering in hibernating bears has been reported (Harlow et al., 2004; Toien et al., 2011), and this activity has been implicated in helping retard muscle atrophy during hibernation (Harlow et al., 2004; Rourke et al., 2006; Lohuis et al., 2007). However, the drastic reduction in weight-bearing activities implies that both the amounts of physiological mechanical loading and neural activation of the muscle are abnormally small. In other mammals, regular neural activation or electrical stimulation of muscles during mechanical unloading is not enough to curtail muscle atrophy (Haddad et al., 2006; Zhang et al., 2010). Thus, it is unclear whether neural activation is enough to retain muscle strength during hibernation.

An alternative hypothesis is that hibernating bears do not need centrally mediated neural activation of muscles to maintain muscle mass and morphology. A rationale for this hypothesis is that the mechanical and neural activities needed for maintaining muscle force-generating properties share many of the same signaling pathways that regulate muscle size. Namely, ubiquitin-mediated pathways for the catabolism of contractile proteins are common for both mechanical and neural-related causes of muscle atrophy (Lecker and Goldberg, 2002; Glass, 2003; Sandri et al., 2006; Zhang et al., 2007). Therefore, an alteration in the muscle trophic signaling pathways during hibernation could allow retention of muscle strength without both mechanical loading and neural activation.

The objective of this study was to test the hypothesis that skeletal muscles of hibernating brown bears are unusually resistant to the

effects of decreased neural activity. Specifically, our aim was to measure the amount of atrophy after denervation of leg skeletal muscles in hibernating bears. Further, we wished to compare that amount to that which occurs in summer-active bears and in other mammalian models in order to determine whether the physiology of hibernating bears has altered the skeletal muscle response to denervation.

MATERIALS AND METHODS

Bear population and hibernation conditions

Eight captive brown bears (*Ursus arctos* Linnaeus 1758; four males, four females) ranging in age from 1 to 20 years old were housed at the Washington State University Bear Research, Education and Conservation Center between fall 2007 and summer 2009. None of the females were pregnant during the study, and all bears had been scheduled previously for euthanasia. Hibernation conditions within this colony have been described previously (Hershey et al., 2008; Nelson et al., 2008). Briefly, bears hibernated from late October to late March singly or in pairs in unheated pens (3×3×2.5 m). In previous years, we have observed in video recordings that bears in this colony were recumbent for 98.2±0.5% of each day between 1 January and 15 February. We assume that the bears included in this study have similar inactivity patterns. During the summer months, bears have access to a 0.56 ha yard for 8–12 h each day and are released in small groups to allow social interaction. Summer activity in this population of captive bears is similar to that observed in wild populations (Rode et al., 2001). The average mass of the bears was 105±42 kg and ranged from 48 to 159 kg at the time of the post-surgical evaluation.

Measurements and the denervation surgery were performed during the second week of December for five hibernating bears and the first week of May for three summer-active bears. These dates were chosen because the span of 11 weeks post-surgery represented the middle portion of the hibernation and summer-active periods. Early and later times are more transitional periods of emergence from and entrance into hibernation (Watts and Cuyler, 1988; Toien et al., 2011).

We focused our measurements on the two main ankle flexor muscles, the cranial tibial (CT; equivalent to the tibialis anterior in humans) and the long digital extensor (LDE), both of which are innervated by the common peroneal nerve. Many previous studies have examined the effects of denervation on plantarflexors of the ankle because of the dramatic changes that occur in anti-gravity muscles (reviewed in Booth and Baldwin, 1995). However, we chose to section the common peroneal, which innervates the dorsiflexors, for three reasons: (1) the surgery was minimally invasive as the nerve is superficial, thus minimizing any local tissue damage (e.g. scarring) or the potential of affecting hibernation behavior; (2) previous studies have measured the seasonal changes in the dorsiflexors of hibernating wild black bears (Harlow et al., 2001; Lohuis et al., 2007), which provides a baseline from which to compare the results of this study; and (3) previous studies in other mammals have shown that denervation of the dorsiflexors produces gross atrophy (Dedkov et al., 2003; Ashley et al., 2007).

Surgical procedures

All animal handling, care and surgical procedures were reviewed and approved by the Washington State University Institutional Animal Care and Use Committee prior to the initiation of the study. The pre- and post-surgical care and handling of the bears have been described previously (Hershey et al., 2008). Briefly, bears were darted with tiletamine HCl and zolazepam HCl (Telazol, Fort Dodge Animal Health, Fort Dodge, IA) to induce anesthesia and were maintained on isoflurane inhalant anesthetic in oxygen *via* an endotracheal tube.

Prior to surgery, ultrasound (US) measurements of whole-muscle dimensions were made in each leg (see below). Following the US measurements, the left leg was shaved and cleaned and a sterile field was maintained. First, an incision (~6 cm in length) was made above the CT and LDE muscles to remove small biopsies (approximately 2×2×1 cm). The biopsies were immediately frozen in isopentane cooled in liquid nitrogen while maintained at their *in vivo* lengths and subsequently stored at –80°C. Second, a 3 cm incision was made 3 cm caudal and 3 cm distal to the head of the fibula. The common peroneal nerve was superficial and easily exposed. To section the nerve, a portion of the nerve (4 cm in length) was removed and latex tubing was sutured around the remaining ends to prevent reinnervation. The procedure was repeated for the right leg, except the nerve was only exposed and not sectioned (i.e. a sham surgery to control for surgical effects). This experimental paradigm is most clinically relevant to a nerve injury and the resulting skeletal muscle atrophy.

All bears immediately after surgery were not able to elevate the front of the foot on the side where surgery had occurred, indicating successful transection of the peroneal nerve. However, within 48 h, all bears were walking with a gait pattern that appeared normal to the untrained observer. General activity was normal, as the bears did not show any abnormal grooming or attention to the foot or surgical areas. The return to normal activities following denervation of dorsiflexors is typical of the response in other quadrupedal mammals, in which locomotor abnormalities can only be detected by detailed gait analysis (Adhihetty et al., 2007; de Ruiter et al., 2007).

After 11 weeks post-surgery, US measurements and biopsies from both legs were obtained again. Biopsies were taken proximal to the previous biopsy sites to avoid possible changes due to the previous biopsy. After the animal was euthanized by injection of pentobarbital (1 ml per 2 kg body mass), the CT and LDE musculotendon complexes of each leg were dissected with their entire proximal and distal tendons. The biopsies and musculotendon complexes were immediately weighed. We also verified that reinnervation of the muscles did not occur by identifying that the ends of the nerve remained separate. The duration of 11 weeks was chosen because the response of skeletal muscles to denervation reaches a steady-state by approximately 11 weeks (see Results).

Measurement of whole-muscle and muscle-fiber morphology

To measure whole-muscle dimensions *in vivo*, we recorded cross-sectional US images of the CT and LDE muscles using a high-frequency linear array transducer (4–13 MHz, MyLab 30, Biosound Esaote, Indianapolis, IN, USA). To standardize the locations of the images and length of the muscles, the knee was fully extended and the ankle was flexed to 90 deg, and the malleolus and tibial plateau were used to measure tibial length. US images were captured just distal to the tibial plateau (i.e. at 10% of tibial length) for the CT and at 50% of tibial length for the LDE because the cross-sectional areas (CSAs) of the muscles were largest at these locations (Fig. 1). Muscle CSAs were determined by hand-drawing an electronic cursor on the periphery of the muscle (MyLab Desk, Biosound Esaote). Anatomical identification of the muscle boundaries was confirmed with comparisons to magnetic resonance images of a bear lower leg used for another study.

To measure average myofiber CSA, we followed the methods of our previous study (Hershey et al., 2008). Briefly, thin sections (10 μm) of the muscle biopsy were ATPase stained (Brooke and

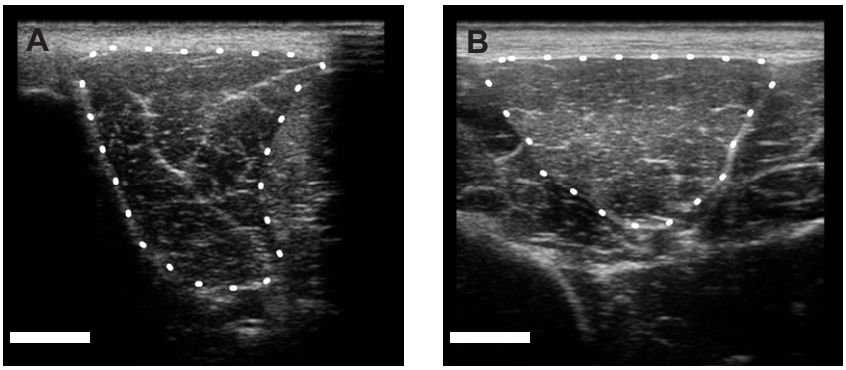

Fig. 1. Ultrasound cross-sectional images of (A) the cranial tibial muscle and (B) the long digital extensor muscle of the brown bear, *Ursus arctos*. Each muscle is outlined with a dotted curve. Scale bars, 1 cm.

Kaiser, 1970) and at least 400 fibers were outlined in a randomized grid with the software NIH Image (National Institutes of Health, Bethesda, MD, USA) (Fig. 2). Fibers were classified as type I (slow) or type II (fast), and no attempt was made to classify fibers within the subtypes of type II.

We compared the denervation responses in hibernating and summer-active bears with those of other mammals, including humans (Castro et al., 1999), laboratory rats (Dedkov et al., 2003), domestic rabbits (Ashley et al., 2007) and guinea pigs (Tomanek and Lund, 1973). Specifically, we compared our results with those of studies that: (1) measured changes in mixed fiber-type muscles (cranial tibial, tibialis anterior or vastus lateralis), because the denervation response is different for these muscles relative to that for postural, primarily slow-type muscles (Booth and Baldwin, 1995); and (2) had within-animal controls to assess the amount of atrophy, which is similar to this study.

Statistical analyses

We calculated the reduction in the three muscle morphology measurements (mass, whole-muscle CSA and myofiber CSA) in the denervated muscles. The reduction was expressed as the percent difference between the measurements from the control and denervated sides relative to the control side (i.e. % difference=(control–denervated)/control×100), such that a positive difference means the denervated side measurement was less than the control side measurement. We also compared the decreases in myofiber and whole-muscle CSA on the treated (denervated) side, expressed as percent difference relative to the pre-surgery measurements. We used a Wilcoxon rank order test to test for a significant difference between the denervation responses of hibernating and summer-active bears. A rank order test was necessary because of the sample size of the population (N=5 for hibernating bears, N=3 for summer-active bears). Results were considered significant at $P<0.05$.

For comparisons between the responses to denervation of fast and slow fibers, we tested for a difference in the amount of CSA reduction between the two myofiber types. We only tested the CT and LDE muscles in the hibernating bear population because of the small sample size of the summer-active bear population. We used the non-parametric two-tailed Wilcoxon signed-rank test (comparable to the parametric paired t-test). Similarly, to determine whether there was a significant decrease in myofiber and whole-muscle CSA between the time of surgery and 11 weeks post-surgery in hibernating bears, we used a one-tailed Wilcoxon signed-ranked test. Results were considered significant at $P<0.05$.

Data are presented as means ± s.d. unless otherwise indicated.

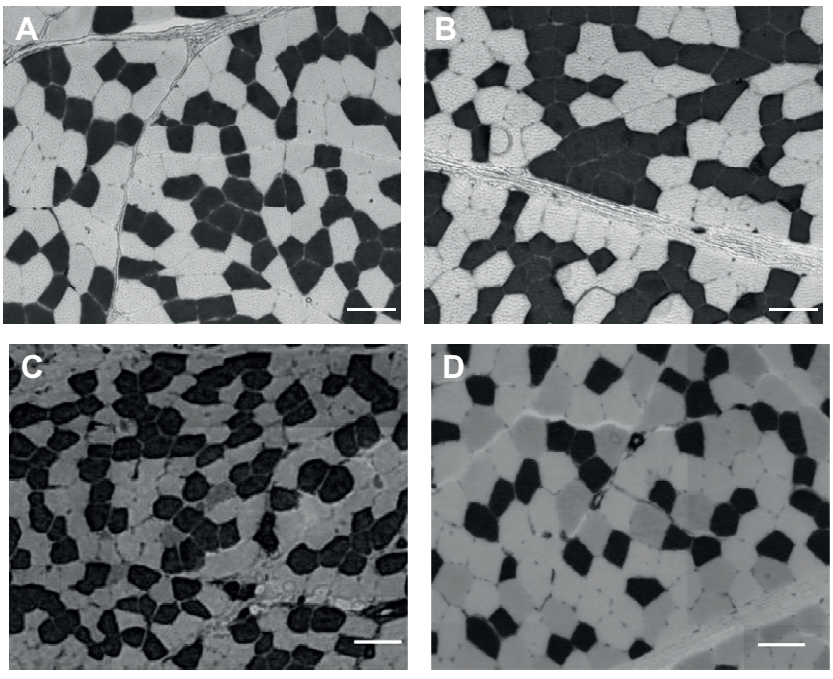

Fig. 2. Representative ATPase stained thin cross-sections of the cranial tibial muscle in a hibernating brown bear obtained 11 weeks post-denervation showing (A) the denervated side and (B) the control (intact) side, and cross-sections taken from a summer-active bear, showing (C) the denervated side and (D) the control side. Slow type I fibers stain dark, and fast type II fibers stain light. Scale bars, 100 μm.

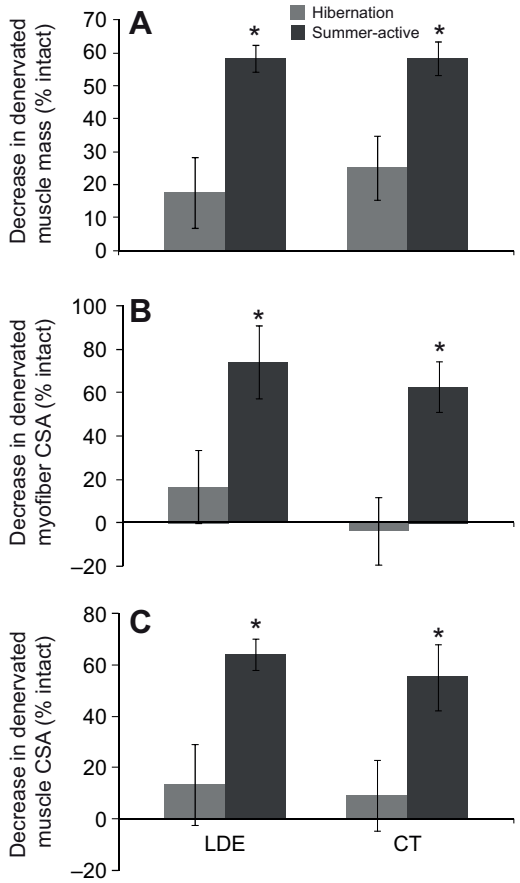

Fig. 3. Average changes in the (A) wet musculotendon mass, (B) myofiber cross-sectional area (CSA) and (C) whole-muscle CSA of the long digital extensor (LDE) and cranial tibial (CT) muscles following denervation for hibernating and summer-active brown bears. Changes are expressed as the percentage difference relative to intact muscle measurements, with a decrease represented as a positive number. Error bars represent ±1 s.d., and asterisks indicate statistically significant differences (P<0.05).

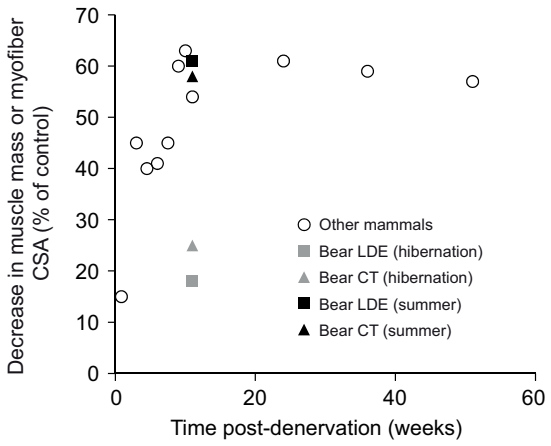

Fig. 4. Comparison of changes in muscle mass or fiber CSA for muscles with mixed fiber types following denervation in other mammals *versus* hibernating and summer-active brown bears. The bear data are the average decreases in musculotendon mass shown in Fig. 3.

RESULTS

In hibernating bears, the average wet musculotendon masses from the denervated side decreased by 18±11% (LDE) and 25±10% (CT) relative to those of the control side (Fig. 3A). In absolute terms, the denervated LDE mass was equal to 70±33 g compared with 84±37 g in the intact leg, and the denervated CT was 39±21 g compared with 52±28 g in the intact leg. In contrast, the average musculotendon masses from the denervated side in summer-active bears decreased by 61±4% (LDE) and 58±5% (CT). These percentages for the LDE and CT corresponded to absolute masses of 54±8 g (denervated) compared with 142.0±33.8 g (intact), and 34±4 g (denervated) compared with 82.1±16.5 g (intact), respectively. The response to denervation was significantly different between seasons (P=0.0357 for both muscles).

The decreases in average whole-muscle and fiber CSAs in the denervated side relative to those in the intact side were similar to those in the wet musculotendon mass. Decreases of 15±16 and 9±14% were found in the LDE and CT whole-muscle CSA, respectively, for hibernating bears, compared with decreases of 64±6 and 49±13%, respectively, for summer-active bears (Fig. 3B). In the hibernating bears, the absolute denervated LDE CSA was 3.4±1.3 cm^2 compared with 4.0±1.6 cm^2 for the intact LDE, and the absolute denervated CT CSA was 2.5±1.7 cm^2 compared with 2.8±1.6 cm^2 for the intact CT. In summer-active bears, the absolute muscle CSA (denervated *versus* intact) was 2.6±0.5 cm^2 compared with 7.3±1.7 cm^2 for LDE, and 2.4±0.4 cm^2 compared with 5.5±1.1 cm^2 for CT.

The average LDE and CT myofiber CSAs decreased by 17±17 and –4±16%, respectively, in hibernating bears, compared with 74±17 and 63±12% in summer-active bears (Fig. 3C). The corresponding absolute values were (denervated *versus* intact): 3330±1101 μm^2 compared with 4172±170 μm^2 (LDE), and 3514±1317 μm^2 compared with 3306±885 μm^2 (CT) in hibernating bears; and 1445±351 μm^2 compared with 6952±3889 μm^2 (LDE), and 1967±211 μm^2 compared with 5576±1536 μm^2 (CT) in summer-active bears. Seasonal responses in both muscles were significantly different (P=0.0357 for both muscles and both CSA measurements).

These changes in musculoskeletal mass and muscle CSA in summer-active bears are very similar to those occurring in other mammals, in which the changes reach a steady-state at approximately 11 weeks post-denervation (Fig. 4). In contrast, the changes during hibernation are approximately one-third those occurring during the summer-active season.

Decreases in LDE and CT whole-muscle CSA relative to the pre-surgery measurements were 22±8 and 21±13%, respectively, in hibernating bears, and 54±4 and 51±10%, respectively, in summer-active bears. Seasonal differences in the decreases were significant in both muscles (P=0.0357). Likewise, decreases in LDE and CT myofiber CSA relative to the pre-surgery measurements were 50±13 and 29±21%, respectively, in hibernating bears, and 70±12 and 61±10%, respectively, in summer-active bears. A seasonal difference in the CT decrease was significant (P=0.0357), but not in the LDE decrease (P=0.0625).

In the LDE of hibernating bears, the decreases in the denervated myofiber CSA were not significantly different between the two fiber types (14±15% decrease in fast fibers *versus* 17±18% decrease in slow fibers; P=1.0; Fig. 5). In the CT, the decreases in the fast and slow fibers were also not significantly different (–2±19% *versus* –6±14%, respectively; P=0.375).

Comparing the pre-surgery and 11 week post-surgery muscle morphology in the intact leg, the decreases in myofiber and whole-muscle CSAs in the LDE of hibernating bears of 19±33% (P=0.188)

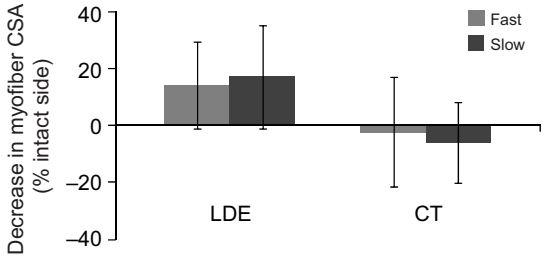

Fig. 5. Average changes in fast and slow myofiber CSAs following denervation for the LDE and CT muscles in hibernating brown bears. No significant differences were found between fiber types.

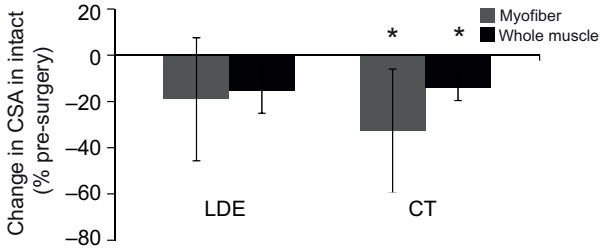

Fig. 6. Average changes in myofiber and whole-muscle CSA in the intact side during the post-surgical period for hibernating brown bears. A negative change represents a decrease in myofiber or whole-muscle size. Asterisks indicate a statistically significant decrease (P<0.05).

and 15±10% (P=0.188), respectively, were not significant (Fig. 6). In contrast, in the CT, the decreases in the myofiber and whole-muscle CSA of hibernating bears of 33±27% (P=0.032) and 14±5% (P=0.032), respectively, were significant.

DISCUSSION

Differences in the effects of denervation between hibernating and summer-active bears

The atrophy that occurred in denervated muscles of the summer-active bears was visibly evident, whereas it was not apparent in the hibernating bears (Fig. 2). Moreover, the fiber and whole-muscle average decreases in CSA were similar in magnitude to the wet musculotendon mass data for both the LDE and CT muscles (Fig. 3). In hibernating bears, this consistency indicates that the musculotendon complex composition and structure did not change drastically, such as in the case of replacement of contractile tissue with connective tissue and/or fat, which can occur following denervation (Tews et al., 1994; Ashley et al., 2007). In summer-active bears, this consistency reflects that most of the muscle mass decrease was due to loss of the contractile components.

Decreases in muscle CSA were calculated relative to the measurements in the control leg to account for intrinsic CSA changes that may have occurred during the period between surgery and euthanasia. However, when muscle CSA decreases were calculated relative to the pre-surgery measurements, seasonal differences were still significant (except for myofiber LDE, for which P=0.0625).

The effect of denervation on skeletal muscle morphology from the summer-active bears was strikingly similar to that in other mammalian models, but was very different during hibernation (Fig. 4). The smaller variability in the summer-active measurements than in the hibernating measurements (Fig. 3) also reflects the stereotyped gross atrophy typical of most mammals. Thus, protection of skeletal muscles from the usual deleterious effects of disuse is not intrinsic to bears year-round and arises from changes in bear physiology occurring during hibernation. Consistent with this finding, gene expression for protein catabolism is downregulated and for protein synthesis is upregulated in skeletal muscle, liver and heart tissues of hibernating black bears relative to summer-active bears (Fedorov et al., 2009; Fedorov et al., 2011).

Another possible explanation for the seasonal differences is that lower metabolic rate during hibernation may have provided protection from atrophy during the denervation. Hibernating bears can reduce overall metabolism by as much as 75% (Toien et al., 2011). In other hibernators, reducing metabolic rate has a muscle-protective effect (Hudson and Franklin, 2002; Biggar and Storey, 2011). However, a protective effect because of reduced metabolism has not been investigated in bears, which maintain a body temperature above 30°C throughout hibernation (Toien et al., 2011), implying that the kinetics for biochemical processes such as proteolysis are not greatly reduced. At this time, we cannot assess how much this reduction in metabolic rate influences the denervation response of skeletal muscles.

Characteristics of changes in muscle morphology following denervation

A consistent finding of denervation studies in other mammals is the selective degradation of slow-type fibers, which are thought to be maintained by having regular low-level neural activation, as in the maintenance of posture (Booth and Baldwin, 1995; Sandri et al., 2006). In hibernating bears, we found no significant difference between fiber type in the amount of atrophy that occurred. This finding was a reflection of the general lack of pronounced atrophy in either fiber type.

A possible explanation for the relatively small amount of atrophy observed in hibernating bears is that gross atrophy could have occurred during the 11 week period because of the inactivity of hibernation, masking the atrophy due to denervation. In the intact leg of hibernating bears, the CSAs were smaller after the 11 week post-surgery period, with the average decrease equaling 14% for muscle CSA and 33% for fiber CSA in the CT (Fig. 6). These data roughly agree with electrical stimulation measurements for wild black bears, where there was a 23% decrease in ankle dorsiflexion force over a 110 day period during hibernation (Harlow et al., 2001). Thus, the normal decreases in muscle CSA in hibernating bears were small relative to the gross changes that were due to denervation as observed in the summer-active bears.

Implications for neurally related pathways regulating muscle morphology and composition

In all mammals, the morphology and composition of skeletal muscle is exquisitely tuned to its functional mechanical demands (Fitts and Widrick, 1996; Bottinelli, 2001). Specifically, muscle size and contractile protein content are usually tightly regulated at all times. Mechanical loading and neural activity are among the primary determinants of muscle state because they reflect the functional use of the muscle (Baldwin and Haddad, 2001; Glass, 2003; Schiaffino et al., 2007). Conversely, when skeletal muscles are not used, they atrophy to conserve metabolic resources, namely to reduce energy consumption and recycle proteins (Jagoe et al., 2002; Sacheck et al., 2007). However, the data presented

here strongly indicate that regulation of skeletal muscle morphology and composition in hibernating bears is not typical of other mammals.

Skeletal muscles of hibernating brown bears are unusually resistant to the effects of eliminating neural activity. Thus, neural activation and/or neurally related trophic factors only play a small role in retaining muscle properties during hibernation. It has been suggested that shivering is a mechanism by which hibernating bats minimize the amount of muscle atrophy (Lee et al., 2010) and could be a mechanism by which disuse atrophy is minimized in hibernating bears (Harlow et al., 2004; Lohuis et al., 2007). A more likely mechanism suggested by this study is that the signaling pathways that regulate the catabolic processes of skeletal muscle are not responsive to the mechanical and neural stimuli that normally control those pathways. Interestingly, analogous observations have been made in the regulation of bone stasis in bears: McGee-Lawrence et al. found that mechanical unloading does not cause bone properties to change during hibernation (McGee-Lawrence et al., 2009).

Signaling pathways regulating skeletal muscle size and composition have not been extensively studied in hibernating bears. In the ventricle in the heart of hibernating bears, mRNA expression of the ubiquitin ligases Muscle Atrophy F-box (MAFBx) and Muscle Ring Finger 1 (MuRF1) did not increase compared with summer-active levels (Barrows et al., 2011). This occurred despite the lower mechanical demands of cardiac muscle during hibernation, with heart rates as low as 9 beats min^{-1} (Toien et al., 2011). The significance of this finding is that the ligases MAFBx and MuRF1 activate the ubiquitin proteosome system and their upregulation leads to muscle atrophy (Bodine et al., 2001; Gomes et al., 2001). In this study, we did not analyze the muscle biopsies for expression levels of signals within either anabolic or catabolic pathways, which might have elucidated the specifics of the alteration in response to denervation that we observed in the hibernating bears.

Because multiple causes of muscle atrophy (e.g. denervation, mechanical unloading and cachexia) with their associated signaling pathways all influence the expression of skeletal muscle ubiquitin ligases, it is not clear which pathway has been altered during hibernation. Alterations in neurally related muscle regulatory pathways relative to other mammals are likely in hibernating bears. More specifically, neural activity is transduced by activation of mitogen-activated protein kinase, calmodulin-dependent protein kinase and/or calcineurin, which in turn acts to increase expression of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) (Bassel-Duby and Olson, 2006; Sandri et al., 2006; Schiaffino et al., 2007). Importantly, PGC-1α inhibits the transcriptional activity of forkhead-related transcription factors, which in turn suppresses the expression of MAFBx and MuRF1 (Sandri et al., 2006). This particular pathway should be a focus for further investigations into the mechanisms of muscle atrophy prevention during bear hibernation.

LIST OF ABBREVIATIONS

CSA	cross-sectional area
CT	cranial tibial
LDE	long digital extensor
MAFBx	Muscle Atrophy F-box
MuRF1	Muscle Ring Finger 1
PGC-1α	peroxisome proliferator-activated receptor γ coactivator 1α
US	ultrasound

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