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REVIEW

Skeletal muscle mass and composition during mammalian hibernation

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ABSTRACT

Hibernation is characterized by prolonged periods of inactivity with concomitantly low nutrient intake, conditions that would typically result in muscle atrophy combined with a loss of oxidative fibers. Yet, hibernators consistently emerge from winter with very little atrophy, frequently accompanied by a slight shift in fiber ratios to more oxidative fiber types. Preservation of muscle morphology is combined with down-regulation of glycolytic pathways and increased reliance on lipid metabolism instead. Furthermore, while rates of protein synthesis are reduced during hibernation, balance is maintained by correspondingly low rates of protein degradation. Proposed mechanisms include a number of signaling pathways and transcription factors that lead to increased oxidative fiber expression, enhanced protein synthesis and reduced protein degradation, ultimately resulting in minimal loss of skeletal muscle protein and oxidative capacity. The functional significance of these outcomes is maintenance of skeletal muscle strength and fatigue resistance, which enables hibernating animals to resume active behaviors such as predator avoidance, foraging and mating immediately following terminal arousal in the spring.

KEY WORDS: Hypometabolism, Torpor, Skeletal muscle, Atrophy, mTOR, PGC-1α

Introduction

When food sources are low and ambient conditions are especially harsh, a number of mammalian species undergo a hypometabolic period with reduced nutrient intake and activity referred to as hibernation. In most animals, this combination of events would result in severe skeletal muscle atrophy accompanied by a shift to faster fiber types more reliant on anaerobic pathways. Yet, hibernating animals do not suffer from profound muscle atrophy, with loss of protein and a shift towards faster fiber types. The following review summarizes skeletal muscle morphological and functional changes occurring during hibernation and delineates the proposed mechanisms behind these changes.

Muscle plasticity in non-hibernators

Mammalian skeletal muscle is an incredibly plastic tissue that remodels itself extensively based on functional demand and nutritional status (Blaauw et al., 2013). Understanding the processes behind skeletal muscle plasticity is important for developing improved training regimes for athletes (Hoppeler et al., 2011), treating various pathological states leading to muscle dysfunction (Doherty, 2003; Tisdale, 2009), and potentially developing novel strategies for reducing muscle loss during long-distance space travel (Blaber et al., 2010). To this end, a number of models have been developed to assess structural changes in skeletal muscle related to activity levels and nutritional status. For example, hindlimb suspension (Thomason and Booth, 1990), bed rest (Widrick et al., 1997) and denervation (Hornberger et al., 2001) are all accepted models for investigating decreased activity and disuse atrophy. Commonalities amongst these models are a decrease in muscle mass (Musacchia et al., 1983; Thomason et al., 1987), protein concentration (Bajotto and Shimomura, 2006; Larsson et al., 1996) and fiber size (Pellegrino and Franzini, 1963; Rittweger et al., 2005; Wagatsuma et al., 2011). Most studies also show a conversion from slow to fast fiber types (Boonyarom and Inui, 2006) and a decline in muscle strength (Adams et al., 2003; Larsson et al., 1996; Thomason and Booth, 1990). Different training regimes can also lead to substantial changes in muscle morphology. For example, increased endurance exercise, characterized by sub-maximal activity for sustained periods of time, leads to marked increases in mitochondrial volume (Hoppeler et al., 1985), capillary density (Waters et al., 2004) and oxidative fibers (Andersen and Henriksson, 1977; Ingjer, 1979), but little change in fiber cross-sectional area (CSA). Conversely, resistance training typically enhances muscle mass, fiber CSA and strength (Andersen and Aagaard, 2000; Carroll et al., 1998; Kosek et al., 2006), with a much smaller shift towards oxidative fast isoforms (Adams et al., 1993; Caiozzo et al., 1996; Campos et al., 2002). In concert with disuse atrophy, skeletal muscle morphology is also strongly affected by nutritional status. During periods of fasting, skeletal muscle mass, fiber CSA, myofibrillar protein and strength (Blaauw et al., 2013; Jagoe et al., 2002; Shindoh et al., 1991) are all reduced.

Muscle plasticity in hibernating animals

Hibernation background

Hibernation is a strategy employed by numerous avian and mammalian species to withstand unfavorable environmental conditions (Ruf and Geiser, 2015). The hibernation state is characterized by long periods of inactivity, depressed metabolic rate, and fasting with increased reliance on fatty acids for metabolic fuel (Andrews, 2007). Most small mammal hibernators cycle through periodic torpor bouts with a duration of 5–40 days (mean 14 days) throughout a winter season (Geiser and Ruf, 1995). Each torpor bout is characterized by almost complete cessation of activity, greatly depressed metabolic rate and a body temperature that can be close to or even slightly below freezing (Geiser, 2004; Geiser and Ruf, 1995). During cyclic arousals, animals expend an incredible amount of energy to regain a normothermic body temperature. This process occurs through both non-shivering and shivering thermogenesis (Choi et al., 2001; Lee et al., 2010), and is characterized by high energy expenditure that approaches the upper limits for sustained aerobic metabolism (Geiser, 2004; Heldmaier et al., 2004; Peterson et al., 1990). Marsupial hibernators are unique in the sense that many species do not exhibit a robust non-shivering thermogenic response (Cortes et al., 2014; Opazo

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et al., 1999; Polymeropoulos et al., 2012), and as such they may be more reliant on shivering thermogenesis during arousal. Because of their much larger body mass and thermal inertia, bears display several differences in hibernation characteristics from those of small mammal hibernators. For instance, bears only reduce body temperature by 2–3°C during their single, uninterrupted bout of winter torpor despite reducing metabolic rate by 75–80% compared with pre-hibernation values (Toien et al., 2011; Watts et al., 1981). Also, bears do not urinate for the entire winter season, potentially eliminating nitrogen loss through the urine, and instead recycle almost 100% of the urea they produce back into endogenous protein (Barboza et al., 1997).

Morphological changes during hibernation

Studies examining skeletal muscle changes in hibernating mammals have been limited primarily to rodents, with fewer studies examining skeletal muscle changes in bats and bears. Despite limited activity and prolonged fasting conditions that typically result in severe muscle atrophy, those species studied appear to be remarkably adept at maintaining skeletal muscle mass during the winter season.

Although muscle mass and protein content are reduced during hibernation in most rodent and bat species studied to date, it is not as profound as that seen in traditional models of disuse or starvation. The majority of studies conducted on hamsters (Deveci and Egginton, 2002b; James et al., 2011), ground squirrels (Cotton and Harlow, 2010; Gao et al., 2012; James et al., 2013; Nowell et al., 2011; Steffen et al., 1991; Yang et al., 2014) and bats (Kim et al., 2000; Lee et al., 2008; Yacoe, 1983a) have shown relatively minor changes in muscle mass and protein content during hibernation, with most reporting 5–30% decreases in mass and 5–15% decreases in protein (Table 1). There is, however, some variation depending on species, specific muscles and sampling period. For instance, the semitendinosus muscle lost up to 60% of mass in hibernating hamsters (Wickler et al., 1987) and 43% of mass in hibernating ground squirrels compared with pre-hibernation animals (Wickler et al., 1991). In contrast, the diaphragm appears to be especially resistant to atrophy, with at least two studies showing an increase in mass during hibernation (Deveci and Egginton, 2002b; Reid et al., 1995). Despite a season-long hypothermia of 2–3°C, bears have very similar alterations in muscle protein to those of small mammal hibernators, with an average loss of muscle protein between 5% and 10% (Table 1) (Hershey et al., 2008; Koebel et al., 1991; Lohuis et al., 2007a; Tinker et al., 1998). Summarizing all studies of mammalian hibernators, post-hibernation values for muscle mass and protein are reduced on average by 15.3% and 7.7% compared with pre-hibernation values and do not seem to be influenced by the size of the hibernating animal or its torpor pattern (Table 1).

In addition to mass and protein measurements, many studies also report fiber CSA changes seen during hibernation. Here again, hibernation typically results in relatively minor changes in fiber CSA, with most studies showing a reduction of only 0–10% (mean change of −4.4% for type I fibers and −2.4% for type II fibers) associated with hibernation (Andres-Mateos et al., 2013; Cotton and Harlow, 2010; Deveci and Egginton, 2002a; Gao et al., 2012; Hershey et al., 2008; Lee et al., 2008, 2010; Malatesta et al., 2009; Tinker et al., 1998). However, at the extremes, a few studies have shown reductions in fiber CSA of between −30% and −55% (Agostini et al., 1991; Steffen et al., 1991). In stark contrast to other models of muscle atrophy (Sandona et al., 2012; Trappe et al., 2004; Widrick et al., 1997), slow oxidative fibers during hibernation do not atrophy more than fast glygolytic fibers. Collectively, these studies indicate that losses of muscle mass, protein and fiber size are typically small during hibernation. Furthermore, the ratio of skeletal muscle mass to body mass is maintained (James et al., 2013; Van Dyke et al., 2007; Yacoe, 1983a) or even improved (Hindle et al., 2014; Yang et al., 2014) following hibernation. Interestingly, animals may emerge from hibernation with greater functional capacity in regards to locomotor performance due to the much greater loss of adipose tissue in relation to skeletal muscle changes.

Hibernators also prevent alterations to muscle fiber type ratio that typically accompany inactivity. Most disuse atrophy models show a profound change towards a greater proportion of fast glycolytic muscle fibers. However, a number of studies on hibernators have shown only a marginal loss of oxidative type I fibers and a retention of fiber ratio throughout the winter (Agostini et al., 1991; Cotton and Harlow, 2010; Deveci and Egginton, 2002a; Nowell et al., 2011; Rourke et al., 2004a; Tinker et al., 1998). Perhaps more surprising are the number of studies showing a moderate increase in type I slow oxidative fiber ratio during the hibernation season despite animals spending 90% of the winter season in an essentially immobile state (Agostini et al., 1991; Gao et al., 2012; Hershey et al., 2008; Lazareva et al., 2012; Malatesta et al., 2009; Nowell et al., 2011; Rourke et al., 2006, 2004a). In fact, hibernators on average increase type I fiber ratio by 25% relative to fiber ratios during the summer active period (Table 1).

Coupled with a strong retention of type I fibers is a preference for fatty acids as a fuel source over carbohydrates (Buck and Barnes, 2000; Hindle et al., 2011). This alteration is accompanied by a decrease in activity and/or concentration of phosphofructokinase (MacDonald and Storey, 2005), fructose 1,6-bisphosphate aldolase (MacDonald and Storey, 2002) and glyceraldehyde 3-phosphate dehydrogenase (Soukri et al., 1995, 1996) during deep torpor. Potential explanations for the decreased activity of glycolytic enzymes include upregulation of pyruvate dehydrogenase kinase 4 (PDK-4; Buck et al., 2002; Glueck and Heldmaier, 2002), decreased binding of enzymes to cytoskeletal elements (Nestler et al., 1997), and low-temperature allosteric changes that inhibit activity (MacDonald and Storey, 2005). Concomitant with decreased rates of glycolysis, hibernators show upregulation in the activity and concentration of proteins associated with lipid catabolism. Glucagon and noradrenaline (norepinephrine) are more effective stimulators of fatty acid mobilization during hibernation (Moreau-Hamsany et al., 1988), while delivery of fatty acids is enhanced by the upregulation of fatty acid binding proteins in both bats and ground squirrels (Eddy and Storey, 2004; Hittel and Storey, 2001). Additionally, citrate synthase activity is enhanced during hibernation in skeletal muscle of bats (Kim et al., 2000).

Skeletal muscle contractile performance during hibernation

Ultimately, retention of skeletal muscle functionality is perhaps best gauged by contractile performance. Far fewer studies have investigated this aspect of hibernation, but given the resistance to morphological changes occurring during hibernation, it is not surprising that there is little loss in muscle strength over the hibernation period. Indeed, average muscle strength following hibernation is only reduced by 12.6% relative to pre-hibernation values. This trend holds for rodents (Cotton and Harlow, 2010; James et al., 2013, 2011), bats (Choi et al., 1998; Lee et al., 2008) and bears (Harlow et al., 2001; Hershey et al., 2008; Lohuis et al., 2007b) (Table 1). Furthermore, these studies show minor changes in contraction time (2.2% less than pre-hibernation values) and relaxation time (1% more than pre-hibernation values) that are generally in agreement with changes in muscle mass, protein and

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Table 1. Summary of skeletal muscle changes during hibernation

	Muscle	Mass	Protein	Type I fiber ratio	Strength	References
Rodentia						
Cricetus cricetus	Psoas	–	−18.3%	+20%	–	Agostini et al., 1991
	Soleus	–	−23.8%	−1.4%	–	Agostini et al., 1991
Mesocricetus auratus	Tibialis anterior	−5.8%	–	−20%	–	Deveci and Egginton, 2002a
		−6.1%	–	–	–	Deveci and Egginton, 2002b
	Extensor digitorum longus	−12.1%	–	–	–	Deveci and Egginton, 2002b
	Soleus	−3.5%	–	–	–	Deveci and Egginton, 2002b
	Diaphragm	+31.3%	–	–	–	Deveci and Egginton, 2002b
	Gastrocnemius	−29.8%*	–	–	–	Wickler et al., 1987
	Semitendinosus	−62.7%*	−12.9%*	–	–	Wickler et al., 1987
Phodopus sungorus	Extensor digitorum longus	−19.8%*	–	–	−20.5%	James et al., 2011
	Soleus	−32.5%*	–	–	+35.5%	James et al., 2011
Glis glis	Quadriceps	–	–	+39.7%	–	Malatesta et al., 2009
Callospermophilus lateralis	Plantaris	−16.6%*	–	+25.9%	–	Nowell et al., 2011
	Soleus	−6.6%	–	–	–	Nowell et al., 2011
	Medial gastrocnemius	−18.9%*	–	+15.4%	–	Nowell et al., 2011
	Lateral gastrocnemius	−15.3%*	–	−16.3%	–	Nowell et al., 2011
	Diaphragm	−0.7%	+4.6%	−8.5%	–	Rourke et al., 2004b
	Gastrocnemius	−29.5%*	+13.6%	−9.6%	–	Rourke et al., 2004b
	Plantaris	−10.7%	+15.3%*	−32.6%	–	Rourke et al., 2004b
	Soleus	−33.9%*	−12.2%	+6.5%	–	Rourke et al., 2004b
	Diaphragm	+19.0%	–	+16.6%	–	Reid et al., 1995
	Gastrocnemius	−14.4%*	+1.4%	–	–	Wickler et al., 1991
	Semitendinosus	−42.5%*	−12.3%	–	–	Wickler et al., 1991
	Soleus	−18.4%	–	–	–	Wickler et al., 1991
	Extensor digitorum longus	−16.4%*	−20.9%*	–	–	Steffen et al., 1991
	Plantaris	−21.0%*	–	–	–	Steffen et al., 1991
	Soleus	−18.5%*	−8.8%	–	–	Steffen et al., 1991
Spermophilus undulatus	Triceps	–	–	+109.1%*	–	Lazareva et al., 2012
	Longissimus dorsi	–	–	+73.2%*	–	Lazareva et al., 2012
	Gastrocnemius	–	–	+54.7%*	–	Lazareva et al., 2012
	Vastus lateralis	–	–	+168.3%*	–	Lazareva et al., 2012
Spermophilus dauricus	Soleus	−1.6%	–	–	–	Yang et al., 2014
	EDL	−8.2%	–	+46.0%*	–	Gao et al., 2012
Ictidomys tridecemlineatus	Soleus	−14.3%	–	–	+14.8%	James et al., 2013
Cynomys leucurus	Extensor digitorum longus	−13.6%	−16.3%	−27.3%	−25.0%*	Cotton and Harlow, 2010
	Soleus	−18.1%*	−13.3%	+0.0%	−15.8%	Cotton and Harlow, 2010
		–	–	+16.0%*	–	Rourke et al., 2006
Cynomys ludovicianus	Extensor digitorum longus	−6.2%	−9.6%	−16.4%	−22.4%*	Cotton and Harlow, 2010
	Soleus	−13.8%*	−11.9%*	+0.0%	−9.7%	Cotton and Harlow, 2010
		–	–	+3.3%	–	Rourke et al., 2006
Chiroptera						
Eptesicus fuscus	Pectoralis	−26.7%*	−10.1%*	–	–	Yacoe, 1983a
Murina leucogaster	Biceps brachii	0.0%	−1.0%	–	−3.8%	Lee et al., 2008
	Pectoralis	−31.7%*	−14.4%	–	–	Kim et al., 2000
	Biceps brachii	–	–	–	−24.2%*	Choi et al., 1998
Carnivora						
Ursus americana	Biceps femoris	–	−8.2%*	+0.7%	–	Hershey et al., 2008
	Vastus lateralis	–	+5.4%	–	−28.9%*	Lohuis et al., 2007a,b
	Biceps femoris	–	–	+73.1%*	–	Rourke et al., 2006
	Gastrocnemius	–	–	+4.7%	–	Rourke et al., 2006
	Tibialis anterior	–	–	–	23.4%	Harlow et al., 2001
	Biceps femoris	–	−10.4%*	−22.3%*	–	Tinker et al., 1998
	Gastrocnemius	–	−4.2%*	−11.9%	–	Tinker et al., 1998
	Extensor hallicus longus	–	−8.1%	–	–	Koebel et al., 1991
	Gastrocnemius	–	−8.1%	–	–	Koebel et al., 1991

*Significant difference from pre-hibernation values.

fiber-type ratios. In sum, this resistance to change should theoretically preserve endurance function in skeletal muscle. However, very few studies have been conducted that measure changes in muscle fatigue resistance due to hibernation. These have shown mixed results with some suggesting no change in fatigue resistance (James et al., 2011), and others showing small to moderate decreases in muscle fatigue resistance (James et al., 2013; Lohuis et al., 2007b).

Potential mechanisms explaining muscle plasticity in hibernators

Muscle activity during arousal bouts

Although activity levels of hibernating animals are clearly depressed compared with levels in summer animals, hibernation is not completely devoid of skeletal muscle use. Hibernating rodents and bats expend an incredible amount of energy to regain normothermic body temperatures during periodic arousal bouts.

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Table 2. Signaling pathways involved in hibernation muscle changes

	Species	Summary	Reference
Pathways regulating muscle mass	*Ictidomys tridecemlineatus*	Phosphorylated SGK-1 ↑	Andres-Mateos et al., 2013
	Ictidomys tridecemlineatus	Phosphorylated Akt ↓, phosphorylated mTOR ↓	Wu and Storey, 2012
		Phosphorylated mTOR ↑, arousal bouts	Wu and Storey, 2012
	Murina leucogaster	Phosphorylated Akt ↓, mTOR ↓	Lee et al., 2010
		Phosphorylated mTOR ↑, arousal bouts	Lee et al., 2010
	Spermophilus richardsonii	Phosphorylated Akt ↓, Akt activity ↓	Abnous et al., 2008
	Ictidomys tridecemlineatus	Phosphorylated Akt ↓	Cai et al., 2004
	Myotis lucifugus	Phosphorylated Akt ↔	Eddy and Storey, 2003
	Spermophilus dauricus	Calpain ↔, calpastatin expression ↑	Yang et al., 2014
	Ictidomys tridecemlineatus	FoxO3 phosphorylation ↑, FoxO3 expression ↑	Wu and Storey, 2014
	Ictidomys tridecemlineatus	FoxO3 phosphorylation ↑	Andres-Mateos et al., 2013
	Myotis lucifugus	FoxO1 expression ↔	Kornfeld et al., 2012
	Spermophilus lateralis	FoxO1 mRNA ↓↔	Nowell et al., 2011
		MAFbx mRNA ↓↔↑	Nowell et al., 2011
	Murina leucogaster	FoxO1 expression ↔	Lee et al., 2010
		MAFbx ↔, MuRF ↔, calpain expression ↔	Lee et al., 2010
	Spermophilus lateralis	MAFbx mRNA ↑	Rourke et al., 2004b
	Myotis lucifugus	Myostatin expression ↓	Kornfeld et al., 2012
	Spermophilus lateralis	Myostatin expression ↓↔	Nowell et al., 2011
	Ictidomys tridecemlineatus	Myostatin expression ↔	Brooks et al., 2011
		Myostatin expression ↑, arousal bouts	Brooks et al., 2011
Pathways regulating fiber type/oxidative capacity	*Ictidomys tridecemlineatus*	PGC-1α expression ↑	Xu et al., 2013
		Phosphorylated AMPK ↑	Xu et al., 2013
		SIRT 1, SIRT 3 expression ↑	Xu et al., 2013
	Callospermophilus lateralis	Phosporylated AMPK ↑	Healy et al., 2011
	Ictidomys tridecemlineatus	PGC-1α expression ↑	Eddy et al., 2005
	Myotis lucifugus	PGC-1α expression ↑	Eddy and Storey, 2003

Signaling pathways regulating muscle mass and fiber type/oxidative capacity are shown.

These arousals depend in part on non-shivering thermogenesis, but also on shivering thermogenesis. For instance, arousing bats depend primarily on non-shivering thermogenesis for rewarming to body temperatures of 10–15°C (Choi et al., 2001), but increasingly rely on shivering thermogenesis between body temperatures of 15 and 37°C (Choi et al., 2001, 1998; Lee et al., 2010). Although evidence on skeletal muscle activity during arousal is largely anecdotal, one study conducted on hibernating bats demonstrated substantial recruitment of skeletal muscle with up to 3× increases in EMG spike amplitude over a period of several hours (Lee et al., 2010) and metabolic rates elevated by up to 10× basal levels (Geiser, 2004; Heldmaier and Ruf, 1992). While arousal bouts are likely necessary to pay off sleep debt (Strijkstra and Daan, 1997), clear metabolic waste products (Zancanaro et al., 1999) and modulate immune system function (Prendergast et al., 2002), the increased activity associated with them may also serve a secondary purpose to periodically increase muscle loading and myoplasmic calcium concentration, both of which may be important for maintaining skeletal muscle mass and avoiding alterations in fiber type associated with low activity during hibernation. While proximal muscles are most heavily involved in shivering thermogenesis (Bell et al., 1992; Meigal et al., 1998), it is unclear to what extent the more commonly studied distal muscles are involved in shivering thermogenesis during arousal, casting some doubt on the notion that shivering thermogenesis during arousal fully explains the maintenance of skeletal muscle during hibernation. In contrast to small mammal hibernators, for instance, bears do not undergo weekly arousal bouts during the hibernation season, yet maintain skeletal muscle to the same extent as small mammal hibernators. However, at least one study has suggested that bears undergo daily bouts of skeletal muscle activity that appear unrelated to thermogenesis but instead function to maintain muscle mass (Harlow et al., 2004).

PI3K/Akt/mTOR

Maintaining muscle mass is largely a balance between protein synthesis and protein degradation. During individual torpor bouts, there seems to be a depression of both protein synthesis and protein degradation. In bears, protein synthesis is depressed during deep torpor compared with that in the summer months, although this is matched by a corresponding depression of protein degradation, leading to a net balance of protein synthesis and degradation (Lohuis et al., 2007a). Similarly, bats have very low rates of both protein synthesis and degradation during torpor bouts, but rates comparable to summer levels during arousal bouts (Yacoe, 1983b). Transcription and translation are also largely suspended during deep torpor, with upregulation during arousal periods (Epperson and Martin, 2002; Van Breukelen and Martin, 2001).

Protein synthesis in skeletal muscle is largely regulated by activating the mammalian target of rapamycin (mTOR), a serine/threonine kinase that mediates increased translation when phosphorylated (Blaauw et al., 2013). Increased mechanical loading of skeletal muscle is one of the most potent stimuli regulating mTOR activity and subsequent protein synthesis. Loading increases insulin-like growth factor I (IGF-I) signaling through phosphoinositide-dependent protein kinase-1 (PI3K) and protein kinase B (Akt) activation, which subsequently leads to phosphorylation of mTOR and increased translation efficiency of myofibrillar proteins (Hornberger, 2011). In addition to IGFs, focal adhesion kinases associated with integrins are also thought to sense load and activate protein synthesis through mTOR and mTOR-independent pathways (Calalb et al., 1995; Gordon et al., 2001; Klossner et al., 2009). Nutrient availability and the energy status of muscle cells can also regulate the mTOR pathway. For example, increased availability of amino acids can activate the mTOR pathway (Jewell et al., 2013; Proud, 2004; Tokunaga et al., 2004), while increased AMP:ATP ratios can activate adenosine

monophosphate-specific kinase (AMPK) and inhibit the mTOR pathway, thereby reducing protein synthesis when energy availability in the cell falls (Tokunaga et al., 2004).

During hibernation, ratios of phosphorylated Akt and mTOR relative to non-phosphorylated molecules are both depressed during hibernation compared with summer values (Lee et al., 2010). In addition, overall enzymatic activity of Akt is substantially suppressed during hibernation compared with summer values (Abnous et al., 2008). Unlike Akt (Eddy and Storey, 2003), levels of phosphorylated mTOR are increased during arousal bouts, supporting the idea that increased muscle protein synthesis occurs during periodic arousal bouts (Lee et al., 2010; Wu and Storey, 2012). This response is not unique to skeletal muscle as both cardiac (Wu and Storey, 2012) and smooth muscle (Talaei, 2014) also show increases in mTOR activity during arousal from torpor. These data are generally in agreement with past studies identifying arousal periods as being crucial for upregulating protein synthesis (Van Breukelen and Martin, 2001). An increase in mTOR activity without concomitant increases in Akt may be explained by activation of mTOR through an alternative pathway: serum- and glucocorticoid-inducible kinase 1 (SGK-1). Total and phosphorylated concentrations of this kinase may increase during hibernation compared with summer values, providing an alternative pathway by which protein synthesis is maintained during hibernation (Andres-Mateos et al., 2013). Further, *sgk1*$^{-/-}$ mice experience substantially more atrophy during disuse and fasting, whereas mice overexpressing the *sgk1* gene were more resistant to disuse atrophy (Andres-Mateos et al., 2013). mTOR activity can also be potentiated independently of PI3K/Akt signaling through focal adhesion kinase (FAK) activity mediated by increased load sensing at integrins (Klossner et al., 2009), a potential scenario occurring during arousal from torpor (Table 2).

FoxO/ubiquitin/proteosome

Myofibrillar protein degradation in muscle cells is primarily driven by the ubiquitin–proteosome system (Murton et al., 2008). During this process, ubiquitin ligases attach ubiquitin to proteins that are in turn degraded within intracellular proteasomes. The efficacy of the ubiquitin–proteosome system is determined by the expression of two major ubiquitin ligases, muscle atrophy f-box (MAFbx) and muscle RING finger 1 (MuRF1) (Bodine et al., 2001). The FoxO family of forkhead transcription factors controls expression of both MAFbx and MuRF1, and therefore myofibrillar protein degradation. Importantly, increased Akt/mTOR activation leads to phosphorylation of FoxO transcription factors with subsequent removal from the nucleus and decreased expression of MAFbx and MuRF1 (Sandri et al., 2004; Stitt et al., 2004), ensuring that when conditions merit accretion of muscle fiber protein, protein catabolism is curtailed.

As protein degradation is greatly curtailed during torpor, there are likely decreases in proteolytic activity as a consequence of either decreased temperature or active suppression via regulation of FoxO transcription factors and downstream targets. Although FoxO1 levels do not change during hibernation (Lee et al., 2010; Nowell et al., 2011), expression of ubiquitin ligases in skeletal muscle is elevated during hibernation (Rourke et al., 2004b). Elevation of ubiquitin ligases in skeletal muscle should enhance protein catabolism. However, several factors may reduce protein degradation in the face of elevated ubiquitin ligase concentrations. First, activity of the ubiquitin–proteosome system is greatly suppressed at the low body temperatures associated with deep torpor (Velickovska et al., 2005), which would greatly reduce the amount of muscle catabolism taking place during torpor bouts. Further, initial degradation of myofibrillar proteins occurs through the actions of calpains, Ca^{2+}-dependent proteases that cause disassembly of Z-discs and associated proteins (Goll et al., 2003). Although calpain expression is not altered by hibernation (Lee et al., 2010; Yang et al., 2014), calpistatin (inhibitor of calpain activity) levels are increased nearly threefold during the hibernation period (Yang et al., 2014), which would greatly decrease the efficiency of the ubiquitin–proteosome system and further reduce protein catabolism (Table 2).

Myostatin

The PI3K/Atk/mTOR pathway can also be profoundly affected by myostatin, a transforming growth factor (TGF) that prevents activation of Akt, and therefore reduces mTOR-induced protein synthesis while concomitantly allowing FoxO transcription factors access to the nucleus, with increased transcription of MAFbx and MuRF1 (Rodriguez et al., 2014). Potential downregulation of myostatin during hibernation may play a role in preserving muscle mass. Indeed, multiple studies have shown decreased levels of myostatin mRNA during hibernation (Kornfeld et al., 2012; Nowell et al., 2011), a situation that should enhance protein synthesis while simultaneously reducing protein degradation. In ground squirrels, muscles exhibiting the least amount of atrophy tend to also have the greatest depression of myostatin and FoxO (Nowell et al., 2011). When examined on a finer scale, however, myostatin levels appear to increase during arousal periods along with downstream transcription factors Smad 2 and Smad 3 (Brooks et al., 2011), suggesting a potential role in increasing protein turnover during interbout arousal periods (Table 2).

Ca^{2+}/AMPK/PGC-1α

PGC-1α (peroxisome proliferator-activated receptor-γ coactivator-1), a nuclear receptor co-regulator, determines muscle fiber-type plasticity by enhancing the gene expression characteristic of slow oxidative fibers (Hood, 2009; Lin et al., 2002). Besides influencing the expression of contractile proteins and mitochondrial biogenesis, PGC-1α also regulates substrate usage by elevating rates of fatty acid oxidation (Vega et al., 2000) and insulin-sensitive glucose uptake in skeletal muscle (Michael et al., 2001). Alterations in skeletal muscle expression and activity of PGC-1α are typically driven by muscle activity cycles. Sustained muscle contraction corresponds to elevated intracellular calcium as well as increases in AMP:ATP ratio, both of which ultimately increase the activity of PGC-1α via calcium–calmodulin-dependent pathways and AMPK pathways, respectively (Hoppeler et al., 2011; Jager et al., 2007). As a result of this regulation, endurance-type training with prolonged periods of muscle activity tends to increase PGC-1α and ultimately the expression of slow oxidative muscle fibers (Baar et al., 2002; Norrbom et al., 2004), while inactivity tends to reduce PGC-1α activity and enhance the expression of fast glycolytic muscle fibers (Nagatomo et al., 2011).

The increase in muscle activity during arousal associated with shivering thermogenesis (Lee et al., 2010) may have an impact on both intracellular Ca^{2+} and AMPK activity, similar to the effects that endurance training sessions have on intracellular Ca^{2+}, AMPK activity and subsequent PGC-1α upregulation (Baar et al., 2002; Norrbom et al., 2004). Indeed, several studies have shown that relative expression of AMPK does increase during hibernation (Healy et al., 2011; Xu et al., 2013). Besides AMPK, other potential regulators of PGC-1α such as the NAD^{+}-dependent protein acetylases (sirtuins) are also upregulated during hibernation (Xu

et al., 2013). With the increases in upstream regulators of PGC-1α, it should not be surprising that PGC-1α mRNA and protein also increase during hibernation (Eddy et al., 2005; Eddy and Storey, 2003; Xu et al., 2013), and that these increases are associated with increased expression of slow oxidative fibers and high mitochondrial density (Table 2).

While arousal periods and associated aerobic activity may be partially responsible for driving increased expression of PGC-1α and maintenance of fiber-type ratios, two studies suggest that arousal periods may not fully explain this phenomenon. Denervation of brown bear (*Ursus arctos*) cranial tibial and long digital extensor muscles in the summer results in profound atrophy over an 11 week period comparable to traditional denervation atrophy models (Lin et al., 2012). But when the same experiment is repeated during the winter, the atrophy response is greatly attenuated, suggesting that periodic muscle activity and loading may not be required for preservation of skeletal muscle during hibernation (Lin et al., 2012). Similarly, when hibernating ground squirrels were sampled at multiple time points throughout the winter, fiber-type transitions had begun prior to initiation of torpor and were largely complete only a few weeks into the hibernating season (Nowell et al., 2011). Furthermore, by manipulating ambient temperatures, the researchers were able to demonstrate that these changes were not influenced by body temperature and degree of shivering thermogenesis during arousal, suggesting that muscle activity during arousal may not fully explain the fiber-type shifts and preservation of muscle mass seen during hibernation (Nowell et al., 2011).

An alternative explanation may involve the increase in circulating free fatty acids associated with fasting and cold exposure that results in increased activity of peroxisome proliferator-activated receptor (PPAR), which in turn alters fiber-type expression (Schuler et al., 2006; Wang et al., 2004). In addition to muscle activity, fasting and cold exposure can increase the expression of PGC-1α. Under these conditions, circulating levels of free fatty acids increase, which in turn triggers the activity of PPARs. PPARs act both as a downstream target of PGC-1α and as an upstream regulator of PGC-1α activity (Schuler et al., 2006). Furthermore, active PPARδ can itself drive expression of slower, more oxidative muscle fibers (Wang et al., 2004). This would account for the early transition to more slow oxidative fibers, independent of the prolonged aerobic activity associated with arousal periods. As animals acclimate to cold exposure and fasting, skeletal muscle also shows an increased capacity for non-shivering thermogenesis. A recently characterized protein, sarcolipin, reduces sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump Ca^{2+} affinity in skeletal muscle and results in increased ATP consumption and heat generation when mice are exposed to cold ambient temperatures (Bal et al., 2012). Increased expression of sarcolipin decreases sarcoplasmic reticulum uptake of Ca^{2+} in cardiac atrial cells and skeletal muscle cells (Babu et al., 2007; Schneider et al., 2013), potentially altering the expression and activity of PPAR and PGC-1α with resulting alterations in slow fiber expression, mitochondrial biogenesis and endurance capacity (Sopariwala et al., 2014). Although no studies have examined sarcolipin expression during hibernation, this protein may also influence fiber-type expression during hibernation.

Conclusions

Hibernating animals spend considerable amounts of time in a state of reduced activity, combined with prolonged fasting, yet experience little of the muscle atrophy predicted by these conditions. Periodic arousals, combined with increased reliance on fatty acids as a metabolic fuel, may explain some of the changes taking place in mTOR activity and PGC-1α expression and the subsequent protection of skeletal muscle morphology and performance. Additionally, novel pathways such as mTOR regulation by SGK-1 also seem likely to curtail muscle atrophy during hibernation. The potential molecular pathways involved have so far only been explored in rodent and bat models. An intriguing question is whether hibernators with different patterns of torpor expression such as bears (prolonged bouts of shallow torpor) and marsupial hibernators (increased reliance on shivering thermogenesis) demonstrate similar alterations in mTOR and PGC-1α pathways during hibernation.

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