Chapter 36
Biochemical Regulation of Carbohydrate Metabolism in Hibernating Bats

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Abstract Glycolysis is the core pathway of carbohydrate metabolism in cells; it is strongly regulated to mediate the use of sugar fuels for energy production (especially when oxygen is limiting) and biosynthesis as well as to allow opposite carbon flow during gluconeogenesis. Control of glycolysis should be a central part of metabolic suppression during torpor. Regulatory enzymes of carbohydrate catabolism (glycogen phosphorylase, 6-phosphofructo-1-kinase [PFK-1], pyruvate kinase, pyruvate dehydrogenase) were evaluated, along with levels of fructose-2,6-P_2, a potent PFK-1 activator, in tissues of little brown bats (*Myotis lucifugus*) comparing aroused and torpor states of winter-collected animals. The data show substantial changes in enzyme activities and properties indicating differential regulation via reversible protein phosphorylation between aroused and torpid states. Torpor also triggered strong increases at the mRNA and protein level of the hypoxia-inducible transcription factor (HIF-1) (that regulates several glycolytic enzymes) in bat skeletal muscle and liver and the study documented for the first time the involvement of microRNA (miR-106b) and antisense RNA in the regulation of a transcription factor in a hibernating species.

36.1 Introduction

The little brown bat (*Myotis lucifugus* Le Conte, 1831) is abundant over the forested areas of Canada and the U.S., ranging north to Alaska and Labrador. This species is a well-known hibernator and multiple aspects of its hibernation ecology,

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physiology, and biochemistry have been studied by many authors (e.g. Thomas 1993; Thomas and Geiser 1997; Speakman and Thomas 2003; Boyles et al. 2007; Townsend et al. 2008; McGuire et al. 2009; Matheson et al. 2010). *M. lucifugus* has also received significant attention recently as a species of concern with respect to two issues: climate change and the white-nosed fungus epidemic (Humphries et al. 2002; Willis et al. 2011; Meteyer et al. 2011).

The profound suppression of metabolic rate during torpor episodes is a prominent feature of hibernation. In *M. lucifugus*, metabolic rate of during torpor may be just 3–4% of basal metabolic rate while euthermic (Geiser 2004). My laboratory has spent many years analyzing the regulation of metabolic rate depression in hibernation and other forms of hypometabolism (for review: Storey and Storey, 2004, 2007, 2010). We have documented conserved regulatory mechanisms including the use of reversible protein phosphorylation to coordinate global metabolic suppression and reprioritize activities of key enzymes and functional proteins so that homeostasis is re-established in the hypometabolic state. Several of our studies have used *M. lucifugus* as a model species. These include differential regulation of mitochondrial genes and antioxidant enzymes during torpor (Eddy et al. 2005, 2006), up-regulation of fatty acid binding proteins (Eddy and Storey 2004), and torpor-responsive changes in signaling by protein kinases including PKA, PKC, Akt, and p38 MAPK (Mehrani and Storey 1997; Holden and Storey 1998; Eddy and Storey 2003, 2007).

Suppression of carbohydrate catabolism via reversible phosphorylation of regulatory enzymes is a prominent feature of animal hypometabolism (Storey and Storey 2004, 2007). This contributes both to global suppression of biosynthesis during torpor and to carbohydrate sparing by some organs that saves glucose for tissues (e.g., brain, erythrocytes) that need this sugar fuel. Studies of the effects of torpor in the jumping mouse, *Zapus hudsonius*, showed coordinated regulation of glycolytic enzymes including glycogen phosphorylase (GP), 6-phosphofructo-1-kinase (PFK-1) and pyruvate kinase (PK) to suppress carbohydrate catabolism in liver (Storey 1987a) and selective inhibition of GP and PFK-1 in other tissues (Storey 1987b). Inhibition of pyruvate dehydrogenase (PDH) also occurred to shut down carbohydrate entry into mitochondrial catabolism (Storey 1989). In all cases the mechanism was covalent modification via phosphorylation or dephosphorylation. Strong PDH suppression also occurred in thirteen-lined ground squirrels, *Ictidomys* (*Spermophilus*) *tridecemlineatus*, during torpor but there was less evidence for phosphorylation of glycolytic enzymes (Brooks and Storey 1992). It has been suggested that small body mass hibernators need stronger controls to suppress metabolic rate during entry into torpor; indeed, small mass hibernators often show Q_{10} values of 3–4 in torpor compared with euthermia whereas larger species show values closer to 2. This might account for the more prominent controls on glycolytic enzymes in liver of *Z. hudsonius* (body mass 12–25 g, maximally 35 g at the start of hibernation) versus *I. tridecemlineatus* (body mass 130–180 g, maximally 220–240 pre-hibernation). The present chapter examines the responses and regulation of glycolytic enzymes and PDH in selected tissues of little brown bats, one of the smallest hibernating species with a body mass of just 6–8 g.

For all of our studies of bat hibernation, my laboratory is deeply indebted to the generosity of Dr. Donald W. Thomas (Université de Sherbrooke), colleague and friend, who allowed me and different students over the years to invade his laboratory in midwinter to collect *M. lucifugus* samples. Don's death in 2009 at too young an age has left a hole in Canadian hibernation research for the impressive ecophysiological studies by his group greatly improved our understanding of the winter energetics and behavior of small hibernators, both bats (e.g. Thomas 1993; Thomas and Geiser 1997; Speakman and Thomas 2003; Humphries et al. 2002) and chipmunks (e.g. Humphries et al. 2003; Landry-Cuerrier et al. 2008). Our bat research is totally Don-derived with animals from his study sites as our guide. His generous access to his laboratory for conducting torpor/arousal studies was always appreciated along with the midnight food runs. I dedicate this paper to Don's memory.

36.2 Materials and Methods

Bats (6–8 g) were collected in mid-January from abandoned mines near Sherbrooke, PQ where they had been hibernating for 3–4 months. Bats were aroused by collection and remained aroused during transport to Université de Sherbrooke. Upon arrival, half of the bats were kept at 23–24°C and remained aroused for 48 h post-collection before being euthanized by cervical dislocation. The others were placed in an environmental chamber at 5°C, allowed to reenter torpor, and sampled after 36–38 h while torpid. Rectal temperatures at sampling were a mean of 5.6°C for torpid bats and 36–37°C for aroused bats. Tissues were rapidly excised, frozen in liquid nitrogen, transported to Carleton University and stored at −80°C.

Preparation of tissue extracts and standard assays for GP, PFK-1, PK, and PDH, as well as the assay method for fructose-2,6-bisphosphate ($F2,6P_2$) were as described in Brooks and Storey (1992). Analysis of HIF-1α protein by immunoblotting, and levels *hif-1α* mRNA, antisense *hif-1α* transcripts, and microRNA 106b by PCR were as in Maistrovski et al. (2012). Data are means ± SEM, $n = 3$–4 samples from different animals. Statistical testing used the Student's *t* test.

36.3 Results and Discussion

The PDH enzyme complex gates the entry of carbohydrate fuels into mitochondrial oxidative reactions. The complex is tightly controlled not only to regulate the catabolism of carbohydrate versus lipid fuels but also to mediate carbohydrate use as a biosynthetic precursor in tissues that do significant synthesis of fatty acids and amino acids. PDH is strongly suppressed not just during torpor in hibernators (Storey 1989; Brooks and Storey 1992) but also during daily torpor

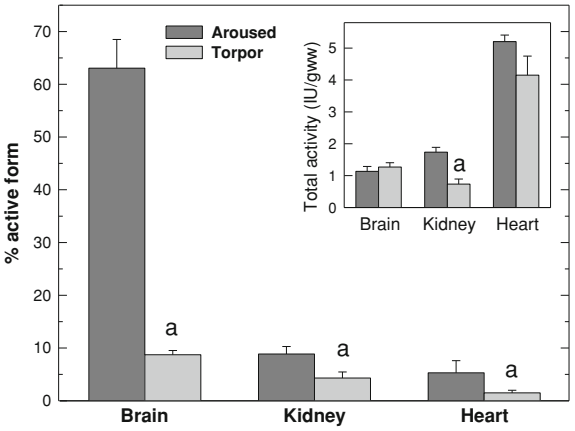

Fig. 36.1 Percentage of pyruvate dehydrogenase (PDH) in the dephosphorylated active a form in tissues from aroused and torpid little brown bats. *Insert* shows total PDH activity determined after in vitro dephosphorylation of the enzyme. *a* significantly different from the corresponding aroused value, $P < 0.01$

(Heldmaier et al. 1999). The major control mechanism of PDH activity is reversible phosphorylation; the enzyme is active in its dephosphorylated form and shut off by phosphorylation via pyruvate dehydrogenase kinase (PDK). Multiple PDK isoforms exist that are responsive to different signals in different tissues; PDK4 is strongly up-regulated during hibernation in ground squirrel heart (Buck et al. 2002). Figure 36.1 shows the strong suppression of PDH in selected bat tissues (those with high aerobic capacity) during torpor. Total activity of PDH (measured after in vitro dephosphorylation of the enzyme) did not change in brain and heart between euthermia and torpor but dropped by 58% in kidney of torpid bats (Fig. 36.1 inset). A similar decrease in total PDH activity of 45% was seen in ground squirrel heart during torpor (Brooks and Storey 1992). However, the major effect of torpor on PDH was a strong decrease in the percentage of total enzyme activity in the active dephosphorylated a form. In bat brain, the percent PDHa dropped from 63% in euthermia to 9% during torpor, kidney PDHa decreased from 9 to 4% and heart PDHa dropped from 5.3 to 1.5%. Similar data were obtained for *Z. hudsonius* (%PDHa decreased from 15% in kidney and 29% in heart to just 1% during torpor in both tissues) (Storey 1989) and *I. tridecemlineatus* (PDH-a activity in kidney and heart of torpid animals was only 3–4% of the euthermic value) (Brooks and Storey 1992).

Another measure of the state of carbohydrate metabolism in tissues is the activity of GP that gives an estimate of the relative dependence on stored glycogen as a fuel. Table 36.1 shows the total activities of GP in six tissues of aroused and torpid bats along with the percentage in the active a form. Total GP activity changed significantly during torpor in only two tissues, increasing by 2.4-fold in heart and decreasing to just 59% of the aroused value in liver. However, the percentage of phosphorylated active GPa changed in all tissues, decreasing by about one-half in liver, brain, kidney, and brown fat whereas % a doubled in skeletal muscle and increased by 1.6-fold in heart. These results for four tissues suggest reduced reliance on carbohydrate fuels during torpor. By contrast, data for

Table 36.1 Total activity of glycogen phosphorylase (measured in the presence of 1.6 mM AMP) and percent in the active a form (measured without AMP) in tissues of aroused and torpid bats

	% a		Total (units/gww)	
	Aroused	Torpor	Aroused	Torpor
Skeletal muscle	20.2 ± 0.9	42.0 ± 4.6[a]	6.05 ± 0.29	5.68 ± 0.68
Heart	20.0 ± 1.4	32.4 ± 6.2[b]	3.63 ± 0.90	8.70 ± 0.37[b]
Liver	84.7 ± 3.2	34.8 ± 4.2[a]	9.60 ± 0.42	5.68 ± 0.75[a]
Brain	65.9 ± 8.3	35.9 ± 3.8[a]	7.00 ± 0.54	6.9 ± 0.21
Kidney	62.5 ± 7.3	38.5 ± 4.0[a]	1.48 ± 0.31	0.95 ± 0.10
Brown fat	55.3 ± 3.9	35.9 ± 1.8[b]	1.73 ± 0.22	2.35 ± 0.44

Significantly different from the corresponding aroused value
[a] $P < 0.01$
[b] $P < 0.05$

heart indicate a prominent increase in dependence on glycogen fuel in heart of hibernating bats; when changes in total GP and %GPa are combined, a nearly fourfold increase in the amount of GPa activity was seen (a rise from 0.73 to 2.82 U/gww). Other species show similar regulation of GP in torpor. Both total GP and %GPa also decreased strongly in *Z. hudsonius* liver giving a net 11-fold reduction in activity during torpor (Storey 1987a) and the amount of active GP also decreased by 60% in torpid brain (Storey 1987b). Liver GPa content decreased by one-half in torpid *I. tridecemlineatus* (Brooks and Storey 1992). Liver is the primary source of carbohydrate fuels for export to organs that depend on glucose; other organs have endogenous reserves of glycogen for their own use but do not export glucose. Hence, it makes sense that glycogenolysis is suppressed in liver during torpor to place strong controls on the consumption of this major glycogen depot. Furthermore, liver is the site of most gluconeogenesis and during torpor would receive significant input of glycerol (derived from triglyceride hydrolysis) for conversion to glucose or glycogen. Inhibition of GP in liver is key to promoting the opposing glycogen synthase reaction.

PFK-1 is the major regulatory enzyme of glycolysis and the ATP-dependent committed step in converting hexose phosphates into triose phosphates for use as aerobic fuels or in biosynthetic reactions. Brigham et al. (1990) found a seasonal decrease in PFK-1 activity (by 60%) in gastrocnemius muscle of hibernating (November) *M. lucifugus* compared with active bats in September correlated with a shift to a higher percentage of fast twitch oxidative fibers in the hibernating season. Seasonal decreases in PFK-1 activity also occurred in pectoralis muscle of *M. lucifugus* and *Eptesicus fuscus* (Yacoe 1983; Brigham et al. 1990). Table 36.2 shows that selected kinetic parameters of liver and skeletal muscle PFK-1 also change significantly over the torpor-arousal cycle (but no differences were found for heart PFK-1). Liver PFK-1 showed significantly reduced affinity for both of its substrates, ATP and fructose-6-phosphate (F6P) during torpor; substrate affinity ($S_{0.5}$) values increased by 67 and 50%, respectively, compared with aroused values. Sensitivity to activation by inorganic phosphate (Pi) was also reduced.

Table 36.2 Kinetic properties of PFK-1 from tissues of aroused and torpid bats

	Liver		Skeletal muscle		Heart
	Aroused	Torpid	Aroused	Torpid	
V_{max} (U/gww)	2.29 ± 0.18	2.11 ± 0.38	2.99 ± 0.38	6.00 ± 0.46[a]	4.90 ± 0.37
$S_{0.5}$ Mg.ATP (μM)	31.0 ± 2.9	51.9 ± 2.1[a]	31.6 ± 0.1	30.7 ± 03.7	22.8 ± 2.4
$S_{0.5}$ F6P (mM)	7.07 ± 0.83	10.6 ± 0.79[b]	2.37 ± 0.18	1.71 ± 0.04[b]	1.74 ± 0.20
I_{50} Mg.ATP (mM)	≫25	≫25	0.78 ± 0.03	0.88 ± 0.09	≫15
I_{50} Mg.citrate (μM)	4,940 ± 580	4,240 ± 490	29.0 ± 1.6	31.8 ± 2.7	134 ± 12
K_a $F2,6P_2$ (nM)	103 ± 20	84 ± 8	33 ± 4	19 ± 2[b]	162 ± 23
K_a AMP (μM)	86.7 ± 1.2	88.3 ± 2.2	13.3 ± 0.84	5.72 ± 0.34[a]	12.5 ± 0.62
K_a Pi (mM)	1.57 ± 0.37	2.88 ± 0.19[a]	0.95 ± 0.06	0.49 ± 0.05[a]	0.68 ± 0.08
K_a NH_4^+ (mM)	3.59 ± 0.25	4.25 ± 0.18	2.16 ± 0.08	2.31 ± 0.40	2.52 ± 0.45

Data for heart are means of aroused and torpid values since no changes were found between the two states for any parameter. $S_{0.5}$ values, [substrate] producing half-maximal velocity, for ATP and F6P were determined at co-substrate levels of 5 mM F6P (10 mM for liver) and 0.5 mM Mg.ATP, respectively. I_{50} is the [inhibitor] that reduced activity by 50% and K_a is the [activator] giving half-maximal activation. For I_{50} ATP determinations, F6P was 3 mM for muscles and 10 mM for liver; I_{50} Mg. was measured at 3 mM F6P (10 mM for liver) and 0.3 mM Mg.ATP. K_a values were determined at 1 mM F6P (2 mM for liver) and 0.3 mM Mg.ATP. Significantly different from the corresponding aroused value

[a] $P < 0.01$

[b] $P < 0.05$

All of these kinetic changes would contribute to inhibiting liver PFK-1 during torpor. The enzyme from *Z. hudsonius* liver also showed altered kinetic properties during torpor that were inhibitory; PFK-1 was less activated by fructose-2,6-P_2 (K_a 2.5-fold higher than controls) and more strongly inhibited by ATP and citrate (I_{50} values were ~fourfold lower than controls) (Storey 1987a). By contrast, skeletal muscle PFK-1 showed changes in kinetic parameters that would generally enhance enzyme function during torpor. Maximal activity increased by twofold, F6P substrate affinity increased ($S_{0.5}$ decreased by 30%) and sensitivity to activation by $F2,6P_2$, AMP and Pi all increased (K_a values reduced by 40–60%). Combined with the data for GP this could suggest a relative shift in favor of carbohydrate catabolism in bat skeletal muscle during torpor. Stable changes in properties of PFK-1 are often due to changes in the phosphorylation state of the enzyme and in vitro incubation studies that stimulated the actions of protein kinases A and C or treated the enzyme with alkaline phosphatase indicated that this was also true of bat skeletal muscle PFK-1. When the K_a $F2,6P_2$ was used as a sensitive measure of PFK-1 modification, incubations of PFK-1 from muscle of aroused bats with cAMP + ATP + PKA or with Ca^{2+} + ATP + phorbol 12-myristate 13-acetate (to stimulate PKC) reduced K_a $F2,6P_2$ by 50–55%, similar to the effect of torpor seen in Table 36.2. By contrast, phosphatase treatment of PFK-1 from torpid bats had the opposite effect, increasing K_a $F2,6P_2$ by 1.9–2.4-fold.

$F2,6P_2$ is the most potent allosteric activator of PFK-1 and is generally considered to regulate PFK-1 with respect to anabolic signals; $F2,6P_2$ typically rises under conditions where carbohydrates are being used for biosynthesis and

decreases under conditions (e.g., hypoxia, starvation) when glycolysis is reserved for energy production (Okar and Lange 1999). $F2,6P_2$ levels rose significantly ($P < 0.05$) during torpor in liver by fivefold (from 0.71 ± 0.16 nmol/gww in aroused to 3.86 ± 0.19 nmol/gww in torpid bats) and in skeletal muscle by 2.6-fold (from 0.50 ± 0.05 to 1.31 ± 0.21 nmol/gww). Levels did not change in other tissues; overall means were 0.58 ± 0.20, 2.85 ± 0.79, 1.36 ± 0.20, and 45.6 ± 1.90 nmol/gww in heart, brain, kidney, and brown fat, respectively. $F2,6P_2$ did not change in either liver or muscle during torpor in *Z. hudsonius* or *I. tridecemlineatus* (Storey 1987a, b). Combined with the opposite decrease in K_a $F2,6P_2$ of skeletal muscle PFK-1 that makes the enzyme more sensitive to K_a $F2,6P_2$ during torpor, this adds further evidence for a relative enhancement of glycolysis in muscle.

The enzyme 6-phosphofructo-2-kinase (PFK-2) synthesizes $F2,6P_2$. PFK-2 was assessed in bat skeletal muscle to determine if altered properties between aroused and torpid states or temperature change might be linked with the altered $F2,6P_2$ levels between the two states. K_m values for F6P substrate did not differ between aroused and torpid states but were ~twofold higher when assayed at 37°C (128 ± 29 vs. 119 ± 33 μM for PFK-2 from aroused vs. torpid bats) than at lower temperatures; values at 25°C were 70 ± 27 and 52 ± 11 μM and at 5°C were 75 ± 25 and 67 ± 13 μM, respectively. Hence, temperature effects in lowering the K_m F6P could help to promote the rise in skeletal muscle [$F2,6P_2$] during torpor.

A previous study of PK from *M. lucifugus* found seasonal differences (early April hibernating vs. August active) in isozyme pattern, activity, and temperature effects on PK from liver and pectoralis muscle that optimized PK for function at lower and variable body temperatures (Borgmann and Moon 1976). Table 36.3 shows that *M. lucifugus* also adjusts PK properties over the torpor-arousal cycle. The bat liver enzyme was differentially regulated between aroused and torpid states and so was heart PK but skeletal muscle PK was not. This latter result agrees with known properties of mammalian muscle PK and a lack of PK modification in both *Z. hudsonius* and *I. tridecemlineatus* muscle during torpor (Storey 1987b; Brooks and Storey 1992). Heart PK showed a 1.7-fold increase in maximal activity during torpor along with a small change in affinity for phosphoenolpyruvate (PEP) substrate and greater inhibition by ATP that could make PK primarily responsive to cellular energy levels during torpor. Bat liver PK showed several significant modifications during torpor. Maximal activity of the enzyme doubled, sensitivity to inhibition by ATP and L-alanine decreased (I_{50} values approximately doubled) and sensitivity to feed-forward activation by $F1,6P_2$ (the product of the PFK-1 reaction) increased (K_a dropped fourfold). All of these changes argue for a more active liver PK in the torpid state although this is contrary to the evidence of less active GP and PFK-1. However, it might be proposed that this data would fit with a model where glycerol derived from triglyceride hydrolysis in other organs was being utilized as a fuel by liver; hence, the triose phosphate portion of glycolysis could be favored while the hexose phosphate portion is relatively suppressed. In vitro incubations with liver PK suggested that reversible phosphorylation was

Table 36.3 Kinetic properties of pyruvate kinase from tissues of aroused and torpid bats

	Liver		Heart		Skeletal muscle
	Aroused	Torpid	Aroused	Torpid	
Vmax (U/gww)	7.53 ± 1.04	16.0 ± 1.37[a]	54.4 ± 8.3	93.2 ± 6.4[a]	53.8 ± 10.0
$S_{0.5}$ Mg.ADP (μM)	410 ± 30	460 ± 30	630 ± 20	660 ± 30	820 ± 40
$S_{0.5}$ PEP (μM)	430 ± 70	480 ± 50	66 ± 11	85 ± 2[a]	56 ± 5
I_{50} Mg.ATP (mM)	19.6 ± 2.2	41.0 ± 6.1[a]	29.1 ± 4.2	19.3 ± 0.2[a]	20.2 ± 3.0
I_{50} L-alanine (mM)	0.37 ± 0.02	0.61 ± 0.05[a]	≫25	≫25	≫50
K_a F1,6P_2 (μM)	4.84 ± 1.50	1.18 ± 0.32[a]	n.a.d.	n.a.d.	n.a.d.

Data for skeletal muscle are means of aroused and torpid values since no changes were detected between the two conditions. $S_{0.5}$ values for ADP and PEP were determined at co-substrate levels of 5 mM PEP and 3 mM ADP, respectively. For I_{50} determinations, PEP was 0.08 mM for muscle and 0.5 mM for liver and heart; for K_a analysis, PEP was 0.02 mM for muscles and 0.20 mM for liver. n.a.d. = no activation detected. Significantly different from the corresponding aroused value

[a] $P < 0.01$

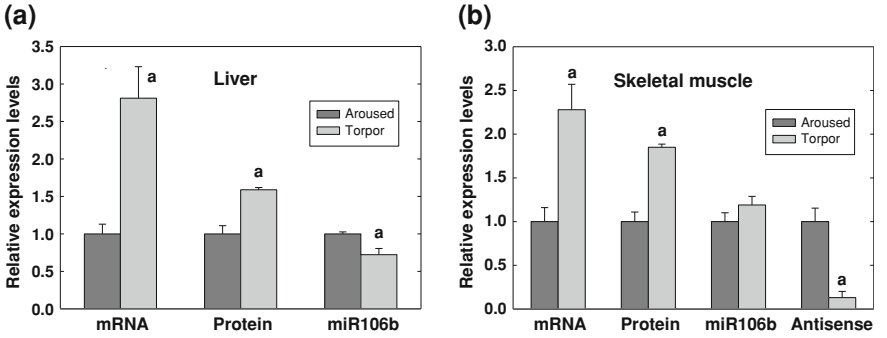

Fig. 36.2 Responses of the hypoxia-inducible transcription factor (HIF-1) system to torpor in *M. lucifugus* liver and skeletal muscle. Relative levels are shown for *hif-1α* mRNA, HIF-1α protein, microRNA 106b, and *hif-1α* antisense RNA. *a* significantly different from the corresponding aroused value, $P < 0.05$. Data compiled from Maistrovski et al. (2012)

responsible for the changes in PK properties between aroused and torpid states. Using I_{50} for L-alanine as an indicator of enzyme modification, the results showed that incubation of PK from aroused bats under conditions that stimulated PKA or PKC action resulted in a 2.1–2.7-fold increase in the I_{50} value, similar to the effect of torpor on the enzyme whereas alkaline phosphatase treatment had no effect (studies with liver of torpid bats were not done). Hence, as for muscle PFK-1, the data for liver PK are consistent with the enzyme from aroused bats being a low phosphate form that is modified by protein kinase action in torpor to create a high phosphate form with altered properties.

Multiple glycolytic enzymes are under transcriptional control by the hypoxia-inducible transcription factor (HIF-1), a dimer made of a constitutively expressed ß subunit and an inducible α subunit that is stable under low oxygen conditions but

targeted for rapid degradation when oxygen is high (Semenza 2007). Previous studies showed HIF-1 involvement in ground squirrel hibernation; HIF-1α protein increased 60–70% in thermogenic organs (brown adipose, skeletal muscle) during torpor and HIF-1 binding to DNA in nuclear extracts increased sixfold indicating greater HIF-1-mediated gene expression during torpor (Morin and Storey 2005). We recently evaluated the HIF-1 system in bat liver and skeletal muscle, finding strong evidence of HIF-1 involvement in torpor (Maistrovski et al. 2012). Figure 36.2 shows that transcript levels of *hif-1α* rose in both tissues (by 2.3–2.8-fold) during torpor compared with aroused bats as did HIF-1α protein (1.6–1.9-fold increase). In a unique discovery about hibernation, HIF-1α expression during torpor was linked with the actions of two kinds of non-coding RNA. In liver, levels of microRNA 106b that negatively regulates *hif-1α* translation were reduced, thereby contributing to increased HIF-1α synthesis during torpor. We previously linked differential expression of other microRNA species with hibernation in *I. tridecemlineatus* (Morin et al. 2008). In muscle, the first evidence of translation control by antisense RNA in hibernation was found. Levels of antisense *hif-1α* RNA decreased sharply during torpor to just 13% of the euthermic value. Antisense transcripts suppress translation by binding to the 3′UTR of a mRNA, both interfering with translation by overlapping ribosome binding sites and recruiting proteins such as the RNA-induced silencing complex (RISC) that targets the mRNA for degradation (Good 2003). Reduced antisense RNA would therefore potentiate *hif-1α* translation to enhance HIF-1α protein levels and lead to selective expression of HIF-1 regulated genes during torpor.

In conclusion, these studies with bats show organ-specific differences in the control of carbohydrate catabolism in torpor versus arousal and key roles for protein phosphorylation in enzyme control and non-coding RNA in HIF-1 regulation.

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