Differential expression of Akt, PPARγ, and PGC-1 during hibernation in bats

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Abstract: The effects of hibernation on the expression of Akt (protein kinase B), the peroxisome proliferator-activated receptor gamma isoform (PPARγ), and the PPARγ coactivator PGC-1 were assessed in seven tissues of the little brown bat, *Myotis lucifugus*. Western blotting revealed that the levels of active phosphorylated Akt were strongly reduced in brain, kidney, liver, and white adipose during torpor as compared with aroused animals and that total Akt protein was also reduced in white adipose during torpor. By contrast, both total and phospho-Akt were elevated in brown adipose tissue, the thermogenic organ. PPARγ and PGC-1 levels showed parallel changes in all organs. Both were strongly suppressed in brain, but levels increased significantly in all other organs during hibernation (except for PGC-1 in heart). Reduced Akt activity is consistent with a probable reduced insulin response during torpor that facilitates the mobilization of lipid reserves for fuel supply and is further supported by increased gene expression of enzymes and proteins involved in lipid catabolism under the stimulation of enhanced PPARγ and PGC-1 levels.

Key words: Myotis lucifugus, mammalian hibernation, lipid metabolism in torpor, protein kinase B, peroxisome proliferator-activated receptor gamma, PPARγ coactivator.

Résumé : Les effets de l'hibernation sur l'expression d'Akt (protéine kinase B), de PPARγ (isoforme gamma du récepteur activé par les proliférateurs des peroxysomes) et de PGC-1 (coactivateur de PPARγ) ont été étudiés dans sept tissus de la petite chauve-souris brune, *Myotis lucifugus*. Des transferts western révèlent que, comparativement aux animaux éveillés, les taux d'Akt phosphorylée active sont grandement réduits dans le cerveau, le rein, le foie et le tissu adipeux blanc durant la torpeur et que la quantité totale de la protéine Akt est également réduite dans le tissu adipeux blanc durant la torpeur. Par contre, les taux d'Akt totale et d'Akt phosphorylée sont élevés dans le tissu adipeux brun, l'organe de la thermogenèse. Les taux de PPARγ et de PGC-1 changent de façon parallèle dans tous les organes. Les deux sont fortement réduits dans le cerveau, mais les taux augmentent significativement dans tous les autres organes durant l'hibernation (sauf celui de PGC-1 dans le cœur). L'activité réduite d'Akt est en accord avec l'hypothèse d'une diminution de la réponse insulinique durant la torpeur qui faciliterait la mobilisation des réserves lipidiques pour les besoins énergétiques de l'animal. Cette hypothèse est également appuyée par l'augmentation de l'expression des gènes codant des enzymes et protéines intervenant dans le catabolisme des lipides, qui est stimulé à la suite de l'augmentation des taux de PPARγ et PGC-1.

Mots clés : Myotis lucifugus, hibernation de mammifères, métabolisme des lipides, torpeur, protéine kinase B, PPARγ, coactivateur de PPARγ.

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Introduction

Many small mammals use hibernation to survive through the long winter months when cold temperatures and little or no available food make normal life impossible. Hibernation is a state of profound metabolic depression; metabolic rate is typically reduced to <5% of the normal, resting rate at 37°C and core body temperature drops to near ambient. By hibernating, animals can conserve as much as 90% of the energy that would otherwise be required to remain euthermic over the winter (Wang and Lee 1996). For hibernating species, the winter season consists of multiple bouts of deep torpor (lasting up to several weeks depending on the species) during which body temperature drops to near ambient. These are interspersed with brief periods of arousal (a few hours or a day) during which euthermia is reestablished. Metabolism during hibernation is largely lipid fueled, making use of huge adipose reserves that are accumulated during summer–autumn feeding. Lipids support basal metabolism during torpor bouts and also fuel the high-intensity thermogenesis during periodic arousals from torpor.

The molecular regulatory mechanisms that are involved in hibernation are a subject of great interest and studies in recent years have made multiple advances on at least three fronts: (*i*) the regulation of metabolic rate depression during the entry into and maintenance of torpor (now known to be largely coordinated by reversible phosphorylation control

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over multiple key loci including enzymes of intermediary metabolism, ion-motive ATPases, and the translational machinery) (Storey 1997, 2001), (*ii*) the readjustment of organ fuel use to support many months of starvation while in torpor (Wang and Lee 1996), and (*iii*) the control of thermogenesis during interbout arousal, particularly the role of uncoupling proteins in brown adipose tissue (Boyer et al. 1998). Progress in all areas has been aided in recent years by gene screening technology that has identified multiple hibernation-responsive genes and proteins and opened several new lines of research (Storey and Storey 2003). For example, the reorganization of fuel use during torpor is supported by increased expression of pyruvate dehydrogenase kinase 4 that helps to suppress carbohydrate catabolism by turning off pyruvate dehydrogenase activity (Andrews et al. 1998) and enhanced expression of fatty acid binding proteins (FABPs) (Hittel and Storey 2001) that support the switch by most organs to lipid fuels in torpor by elevating intracellular fatty acid transport capacity.

Recently, a number of protein kinases, phosphatases, and transcription factors have been implicated in both metabolic and gene expression events associated with hibernation (Frerichs et al. 1998; MacDonald and Storey 1998, 1999; Carey et al. 2000; Ono et al. 2001; Chen et al. 2001; Lee et al. 2002). Multiple controls are needed to initiate and coordinate a wide range of cellular events to allow cells and organs to make smooth transitions to the hypometabolic, hypothermic state. Our current interest is in the signaling mechanisms that regulate the use of carbohydrate versus lipid fuels and that mediate the switch to a lipid-based metabolism by most organs, resulting in drop in whole-animal respiratory quotient to ~0.7 during torpor.

The present study compares the responses during torpor and arousal by Akt (also known as protein kinase B), the peroxisome proliferator-activated receptor gamma (PPARγ, a ligand-activated transcription factor), and PGC-1 (a PPARγ coactivator) in seven tissues of the little brown bat, *Myotis lucifugus*. *Myotis lucifugus* is one of the smallest hibernating species. The metabolic rate of these bats during torpor may be as low as 1–1.5% of the resting basal metabolic rate (Hock 1951) and the hibernating season can last as long as 8–9 months in Canada; hence, the metabolic regulation of fuel use must be very precise to ensure animal survival. Akt is central to insulin signaling and cellular homeostasis of carbohydrate levels in healthy cells (Summers and Birnbaum 1997; Smith 2002); it is also one of several signaling molecules whose activity increases during muscle exercise (Sakamoto and Goodyear 2002; Sakamoto et al. 2002). A recent study of the responses of signaling molecules in brain of a temperate bat (*Rhinolpopus ferrumequinum*) showed a three-fold activation of Akt during the period of high-intensity oxygen consumption and thermogenesis accompanying interbout arousal (Lee et al. 2002). This suggests that Akt levels may be suppressed during torpor, contributing to the overall suppression of carbohydrate catabolism in the hibernating animal. By contrast, the relative utilization of lipid fuels rises during hibernation. Hibernation-induced changes in lipid-related gene and protein expression (e.g., enhanced FABP expression) may be coordinated by transcriptional activators of these genes, including PPARγ and its coactivator PGC-1. The data presented here support these proposed roles for these signal transduction modules in hibernation.

Materials and methods

Animals

Little brown bats (7–8 g body mass) were collected by Dr. D. Thomas on November 30, 1999, from a disaffected slate mine near Sherbrooke, Que., where they had been hibernating since October (cave air temperature was 5°C). Collection aroused the bats and they remained aroused during transport to the Université de Sherbrooke. Upon arrival, 10 bats were maintained under euthermic conditions at 23–24°C air temperature; these were kept awake for a total of 48 h postcollection and were then euthanized by cervical dislocation. Ten others were placed in a cold room at 5°C and allowed to enter torpor; 10–12 h was required for full torpor to be reestablished with a body temperature close to ambient. Animals remained torpid until sampled 36–38 h later; measured rectal temperatures at sampling were 5–6°C. Tissues from euthermic and hibernating animals were quickly excised, immediately frozen in liquid nitrogen, and then transported to Ottawa, Ontario, where they were stored at –80°C until analysis. Protocols for animal care met the guidelines of the Canadian Council on Animal Care.

Tissue homogenization and protein preparation

Frozen tissue samples were homogenized in 100 mM MOPS, 25 mM HEPES, 25 mM β-glycerophosphate, 5 mM EDTA, 1 mM EGTA, and 250 μM $NaVO_4$, pH 7.4, with 1 mM phenylmethylsulfonyl fluoride added immediately before homogenization. Soluble protein content was measured using the BioRad prepared reagent and then samples were diluted 1:1 in 2× sodium dodecyl sulfate sample buffer. Aliquots containing 15 μg of total protein were loaded into each lane and electrophoresis and transfer to PVDF membranes (Biotrace, PALL Life Sciences) was carried out as described previously (Hittel and Storey 2002).

Antibodies

Akt and phospho-Akt (Ser 473) antibodies were purchased from Cell Signaling Technology, Inc., PPARγ antibody was purchased from Cedarlane Labs (Hornsby, Ont.), and PGC-1 antibody was a gift of Dr. Dan Kelly (Washington University, St. Louis, Mo.). Antibody dilutions of 1:1000 in TBST were used for all procedures. Secondary rabbit IgG HRP-conjugated antibody was purchased from Cell Signaling. Blots were developed using the Western Lightning™ Chemiluminescense Plus (NEN, Perkin Elmer) system according to protocols provided with the system. Blots were briefly exposed to Kodak X-OMAT-AR film and developed according to manufacturer's protocols. X-ray images were quantified using densitometric analysis software (Imagequant, Molecular Imaging). After antibody reactions were complete, blots were stained with Coomassie blue to confirm equal loading.

Data and statistics

Owing to the very small sizes of most bat organs, frozen tissue samples were combined and ground to a powder under

liquid nitrogen with a mortar and pestle. Two pooled tissue sources were prepared originating from 10 aroused and 10 torpid bats. All data are from three independent isolations from the pooled tissue. Ratios of torpor versus aroused values were calculated as follows. The band intensity of immunoreactive material in each lane of each blot was first normalized against corresponding Coomassie-stained protein bands in the same lane that did not change between torpor and arousal. Mean normalized band densities ± SEM for aroused versus torpid samples were then calculated and significant differences between the groups were tested using Student's *t* test. The ratio of torpor to arousal was then plotted; error bars on the final histograms are the sum of SEM values for torpor and arousal trials.

Results

Total Akt and phospho-Akt levels in bats

Western blotting detected a single band that cross reacted with either Akt or phospho-Akt antibodies at the expected molecular weight for Akt of 60 kDa. Figure 1 shows the relative amounts of total Akt protein and phosphorylated Akt content in aroused and torpid bats. Akt in brown adipose tissue was strongly affected by torpor. Total Akt protein content was 2.5 ± 0.7-fold ($P < 0.05$) higher in torpid bats compared with aroused bats. The relative amount of active phospho-Akt was also 2.9 ± 0.6-fold ($P < 0.05$) higher in brown adipose during torpor than during arousal. Total Akt protein content did not change between aroused and torpid states in brain, kidney, or liver but was significantly lower in white adipose tissue during torpor (torpor to aroused ratio was 0.3 ± 0.2, $P < 0.05$). However, the amount of phosphorylated Akt was significantly lower in all four of these organs during torpor with torpor to aroused ratios of 0.3 ± 0.2 ($P < 0.001$) in brain, 0.3 ± 0.1 ($P < 0.05$) in kidney, 0.4 ± 0.1 ($P < 0.001$) in liver, and 0.4 ± 0.1 ($P < 0.01$) in white adipose tissue during hibernation. Neither total nor phospho-Akt content was affected in heart or skeletal muscle.

PPARγ protein expression

PPARγ is important in the regulation of genes involved in lipid catabolism and we predicted that levels of this factor would rise in hibernator organs to promote the increased use of stored lipid reserves as fuels during torpor. Relative PPARγ levels were assessed by Western immunoblotting; a single band of cross-reacting material at the expected molecular weight of 55 kDa was detected in all organs. Figure 2 shows the ratio of PPARγ levels in torpid versus aroused states for the seven tissues. PPARγ protein levels were significantly higher in six organs of torpid bats as compared with aroused animals. In particular, PPARγ was threefold higher in brown adipose of torpid bats and 2.6-fold higher in skeletal muscle. PPARγ levels were 1.4- to 2.0-fold higher during hibernation in heart, liver, kidney, and white adipose. By contrast, PPARγ levels were reduced in brain during torpor to a torpor to aroused ratio of 0.6.

PGC-1 protein expression

PGC-1 can act as a coactivator of PPARγ and its expression often correlates well with that of PPARγ. The present data support this idea and show a strong correlation between PGC-1 and PPARγ levels that is both qualitative and quantitative. A band cross reacting with the PGC-1 antibody was detected in all organs at the expected molecular weight of 85–90 kDa. Densitometric quantification showed that PGC-1 levels were higher in five organs of torpid animals as compared with euthermic aroused bats, and the greatest increase in PGC-1 content was again a threefold higher amount found in brown adipose of torpid animals (Fig. 2). PGC-1 contents were 1.8- to 2.3-fold higher in kidney, liver, skeletal muscle, and white adipose during torpor but PGC-1 content in heart showed no significant differences between the two states. As for PPARγ, PGC-1 content in brain was significantly lower during torpor with a torpid to aroused ratio of 0.6.

Discussion

The data presented here show differential expression of Akt, PPARγ, and PGC-1 in multiple organs of hibernating little brown bats. In general terms, three categories of response by Akt were seen during torpor as compared with animals that were aroused: (*i*) higher amounts of both total and phospho-Akt content in brown adipose, (*ii*) no change in Akt expression in heart and skeletal muscles, and (*iii*) reduced amounts of active, phospho-Akt in brain, kidney, liver, and white adipose, the effect in white adipose being compounded by a much lower total Akt content. PPARγ and PGC-1 responses were coordinated in all tissues and two general categories of response emerged: (*i*) levels of both were higher in six organs during torpor (with the exception of PGC-1 in heart) and (*ii*) levels of PPARγ and PGC-1 were significantly lower in brain during hibernation compared with the aroused state. The reduced levels of all three signal transduction elements in brain are consistent with the very strong suppression of metabolic and neurologic functions in hibernator brain (Frerichs et al. 1998; Chen et al. 2001). The lower levels of phospho-Akt in the brain of hibernating *M. lucifugus* and higher levels in aroused bats also correlate well with a recent report on brain of another bat species where it was shown that Akt levels rose during arousal from torpor (Lee et al. 2002).

The changes in Akt, PPARγ, and PGC-1 in bat tissues are generally consistent with the known patterns of change in fuel use during mammalian hibernation. Overall, carbohydrate use by organs is strongly suppressed during torpor; for example, the activity of pyruvate dehydrogenase that gates carbohydrate entry into the tricarboxylic acid cycle can be reduced during torpor to just 3–4% of euthermic values (Storey 1997, 2001). Carbohydrate reserves are largely preserved for use as a fuel for the brain. The relative consumption of lipids as fuels increases strongly in virtually all organs; even the brain becomes lipid dependent after a fashion because it derives a significant percentage of its energy from the oxidation of ketones. Clearly, these patterns of fuel use in hibernation are also those that are characteristic of starvation in mammals, and although some modifications probably occur that are related to the low body temperature

Fig. 1. Effects of torpor on total and phosphorylated levels of Akt in *M. lucifugus* tissues. Representative Western blots show total Akt protein and phospho-Akt levels in samples from aroused (A) versus torpid (T) bats. Antibodies detected a dominant band at 60 kDa that was determined to be Akt. Mean ± SEM values for normalized band intensities were calculated from $n = 3$ trials for both aroused and torpid samples followed by significance testing using Student's t test; *torpor value is significantly different from aroused value ($P < 0.05$). Mean values for the torpor to aroused ratio were then calculated and are presented in the histogram. BAT, brown adipose tissue; WAT, white adipose tissue.

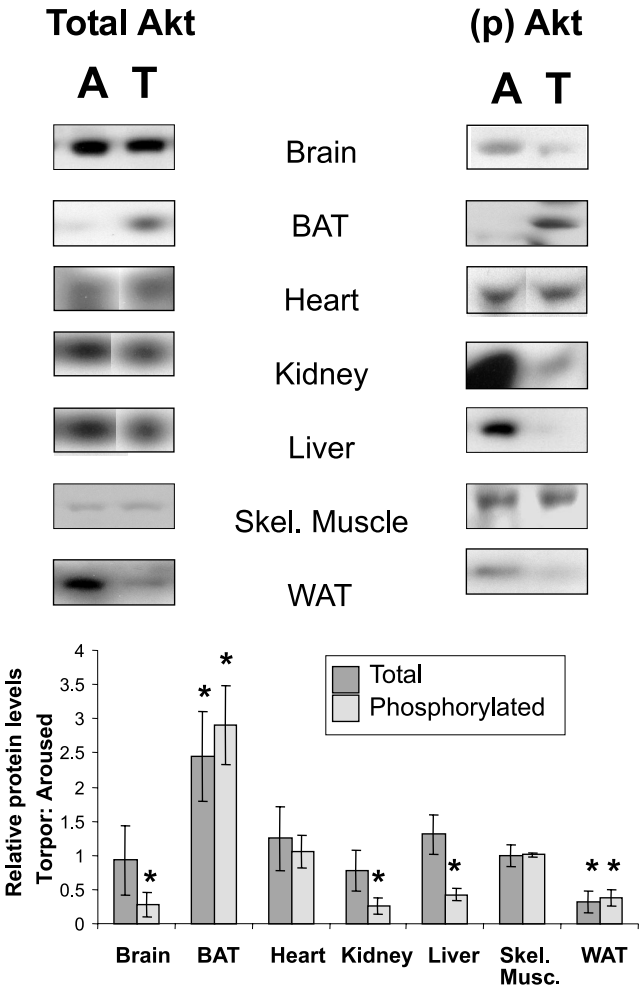
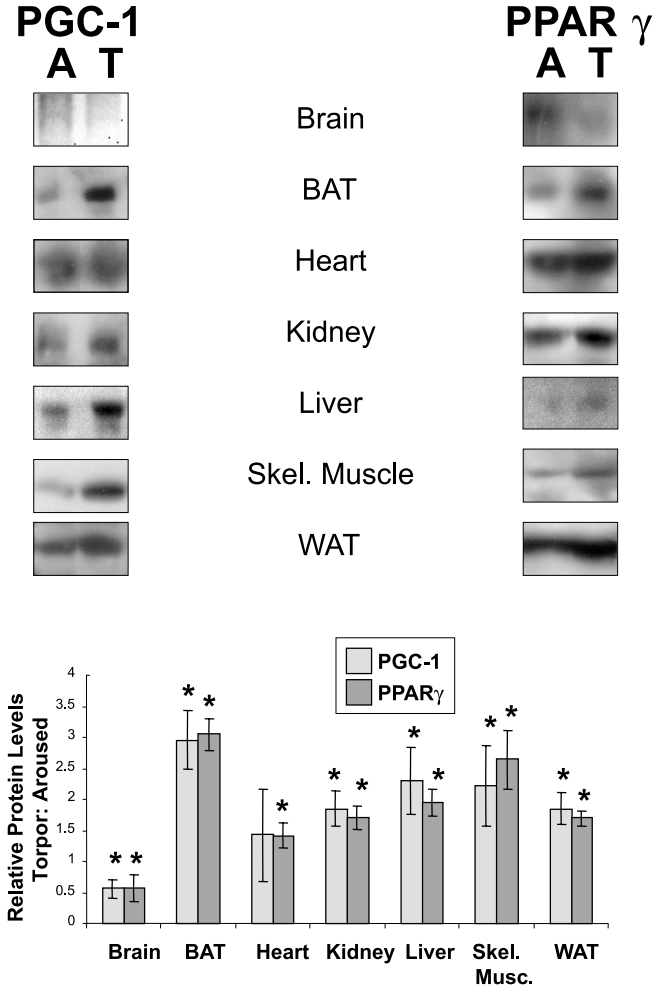


Fig. 2. Effects of torpor on PGC-1 and PPARγ protein expression. Representative Western blots show total protein levels in samples from aroused (A) versus torpid (T) animals. The PGC-1 antibody detected a band between 85 and 90 kDa. The PPARγ antibody detected a single band at 55 kDa. Other information as in Fig. 1.



and the deep torpor of hibernation, the regulatory mechanisms that control fuel use in hibernation are proving to be basically the same as the mammalian model for starvation.

The present data on Akt, PPARγ, and PGC-1 are consistent with this view. Akt is part of the insulin-signaling pathway that promotes carbohydrate storage as glycogen and carbohydrate use for fatty acid biosynthesis in the fed state. For example, Akt activation is linked with increased glucose uptake into muscle cells, likely due to stimulation of the glucose transporter GLUT4 (Summers and Birnbaum 1997). Akt phosphorylation of glycogen synthase kinase 3 inhibits the enzyme and prevents it from phosphorylating and inactivating glycogen synthase, thereby promoting glycogen synthesis. The reduced (or unchanged) amounts of active phospho-Akt in several bat organs are consistent with reduced insulin signaling during torpor. Indeed, both insulin secretion from the pancreas and plasma levels of the hormone are strongly reduced in most hibernators (Wang and Lee 1996; Bauman et al. 1987). Very little is known about carbohydrate and lipid metabolism in hibernating bats. One study of *M. lucifugus* showed that pancreatic levels of insulin increased during deep torpor (Bauman 1990); the author hypothesized that this did not represent circulating insulin in the blood plasma during torpor but rather created a storage pool of insulin that could be secreted rapidly upon arousal. This study also noted high levels of glucagon during hibernation in *M. lucifugus* that may be required to regulate the use of stored carbohydrate reserves. Another study investigated lipid responses in hibernating bats; data on the big brown bat, *Eptesicus fuscus*, showed that during arousal from torpor, plasma free fatty acids were rapidly mobilized (Fonda et al. 1983).

Our data correlate well with known parameters of fuel metabolism in hibernating species including the cessation of

biosynthesis of new fuel reserves over the winter months, a strong decrease in the activities of enzymes of fatty acid synthesis in liver (e.g., fatty acid synthase activity falls to about 10% of the euthermic value; Frank et al. 1998), and a reorganization of fuel metabolism for a regulated slow catabolism of lipid reserves (with general carbohydrate sparing) over the months of hibernation. However, the response of Akt in brown adipose tissue was novel, with both total Akt and phospho-Akt contents being much higher in the tissue from hibernating versus aroused animals. At present the function of high Akt activities in this organ during torpor is unknown and it presents an intriguing problem for future study. Interestingly, however, exactly the opposite response occurred in white adipose, which was the only tissue to show much lower levels of both total Akt and phospho-Akt during torpor. The response in white adipose tissue is certainly what is expected from the decrease in insulin signaling, which would suppress triglyceride synthesis and storage in white adipose in favour of triglyceride catabolism when hibernation begins.

PPAR transcription factors promote the expression of genes involved in fatty acid transport and metabolism and the coactivator PGC-1 stimulates their function (Lowell 1998). Notably, transcription of PGC-1 itself is inhibited by insulin and it is one of the factors that provides reversible control over fatty acid biosynthesis versus oxidation. Higher levels of both PPARγ and PGC-1 during torpor, compared with arousal, in all bat organs (except brain) suggest an overall up-regulation of genes involved in lipid catabolism during hibernation including FABPs. Both the adipose and heart isoforms of FABP are strongly up-regulated during hibernation in multiple tissues of both ground squirrels (Hittel and Storey 2001) and bats (S.F. Eddy, unpublished data) and PPARγ is known to increase the expression of adipose FABP in other mammals (Berger and Moller 2002). In addition, insulin-sensitive rats show a marked increase in heart FABP expression when fed PPARγ agonists (Way et al. 2001). Two other known targets of PPARγ in mammalian adipose tissue are pyruvate dehydrogenase kinase 4 and the mitochondrial uncoupling protein; the latter is key to thermogenesis by brown adipose tissue (Berger and Moller 2002). Both are known to be up-regulated during hibernation (Boyer et al. 1998; Andrews et al. 1998), and indeed, our data show that the largest fold increase in PPARγ and PGC-1 levels in hibernating *M. lucifugus* was observed in brown adipose.

The bats under study had been hibernating for about 36 h after a 12-h euthermic interval, so it could be proposed that the elevated levels of PPARγ and PGC-1 in organs from torpid bats stimulated renewed gene expression and synthesis of selected proteins that are key for survival in torpor and for the next arousal, proteins that may have been depleted or damaged during the euthermic interval. Notably, brain was the one organ that showed suppressed PPARγ and PGC-1 during hibernation, but brain does not utilize fatty acid fuels. It is also interesting to note that the gamma isoform of PPAR is typically described as being abundant in adipose tissue and low in other tissues of nonhibernating mammals (Berger and Moller 2002). However, this is not true in bats, as the transcription factor was found in all seven tissues tested. This may attest to an enhanced importance of PPARγ and PGC-1 in the regulation of metabolism in hibernating species.

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References

Andrews, M.T., Squire, T.L., Bower, C.M., and Rollins, M.B. 1998. Low-temperature carbon utilization is regulated by novel gene activity in the heart of a hibernating mammal. Proc. Natl. Acad. Sci. U.S.A. **95**: 8392–8397.

Bauman, W.A. 1990. Seasonal changes in pancreatic insulin and glucagon in the little brown bat (*Myotis lucifugus*). Pancreas, **5**: 342–346.

Bauman, W.A., Meryn, S., and Florant, G.L. 1987. Pancreatic hormones in the nonhibernating and hibernating golden mantled ground squirrel. Comp. Biochem. Physiol. A. Comp. Physiol. **86**: 241–244.

Berger, J., and Moller, D.E. 2002. The mechanisms of action of PPARs. Annu. Rev. Med. **53**: 409–435.

Boyer, B.B., Barnes, B.M., Lowell, B.B., and Grujic, D. 1998. Differential regulation of uncoupling gene homologues in multiple tissues of hibernating ground squirrels. Am. J. Physiol. **275**: R1232–R1238.

Carey, H.V., Frank, C.L., and Seifert, J.P. 2000. Hibernation induces oxidative stress and activation of NK-kB in ground squirrel intestine. J. Comp. Physiol. B. Biochem. Syst. Environ. Physiol. **170**: 551–559.

Chen, Y., Matsushita, M., Nairn, A.C., Damuni, Z., Cai, C., Frerichs, K.U., and Hallenbeck, J.M. 2001. Mechanisms for increased levels of phosphorylation of elongation factor-2 during hibernation in ground squirrels. Biochemistry, **40**: 11 565 – 11 570.

Fonda, M.L., Herbener, G.H., and Cuddihee, R.W. 1983. Biochemical and morphometric studies of heart, liver and skeletal muscle from the hibernating, arousing and aroused big brown bat, *Eptisicus fuscus*. Comp. Biochem. Physiol. B. Comp. Biochem. **76**: 355–63.

Frank, C.L., Brooks, S.P.J., Harlow, H.J., and Storey, K.B. 1998. The influence of hibernation patterns on the critical enzymes of lipogenesis and lipolysis in prairie dogs. Exp. Biol. Online, **3**: 9.

Frerichs, K.U., Smith, C.B., Brenner, M., DeGracia, D.J., Krause, G.S., Marrone, L., Dever, T.E., and Hallenbeck, J.M. 1998. Suppression of protein synthesis in brain during hibernation involves inhibition of protein initiation and elongation. Proc. Natl. Acad. Sci. U.S.A. **95**: 14 511 – 14 516.

Hittel, D., and Storey, K.B. 2001. Differential expression of adipose- and heart-type fatty acid binding proteins in hibernating ground squirrels. Biochim. Biophys. Acta, **1522**: 238–243.

Hittel, D., and Storey, K.B. 2002. The translation state of differentially expressed mRNAs in the hibernating thirteen-lined ground squirrel (*Spermophilus tridecemlineatus)*. Arch. Biochem. Biophys. **401**: 244–254.

Hock, R.J. 1951. The metabolic rates and body temperatures of bats. Biol. Bull. **101**: 289–299.

Lee, M., Choi, I., and Park, K. 2002. Activation of stress signaling molecules in bat brain during arousal from hibernation. J. Neurochem. **82**: 867–873.

Lowell, B.B. 1998. Adaptive thermogenesis: turning on the heat. Curr. Biol. **8**: R517–R520.

MacDonald, J.A., and Storey, K.B. 1998. cAMP-dependent protein kinase from brown adipose tissue: temperature effects on kinetic properties and enzyme role in hibernating ground squirrels. J. Comp. Physiol. B. Biochem. Syst. Environ. Physiol. **168**: 513–525.

MacDonald, J.A., and Storey, K.B. 1999. Regulation of ground squirrel Na^+ K^+-ATPase activity by reversible phosphorylation during hibernation. Biochem. Biophys. Res. Commun. **254**: 424–429.

Ono, M., Hosoe, Y., Azuma, S., Shoji, M., Nara, K., Kondo, N., Shiba, T., and Takamatsu, N. 2001. HNF-1 regulates the liver-specific transcription of the chipmunk HP-20 gene. Gene, **277**: 121–127.

Sakamoto, K., and Goodyear, L.J. 2002. Invited review: intracellular signaling in contracting skeletal muscle. J. Appl. Physiol. **93**: 369–383.

Sakamoto, K., Hirshman, M.F., Aschenbach, W.G., and Goodyear, L.J. 2002. Contraction regulation of Akt in rat skeletal muscle. J. Biol. Chem. **277**: 11 910 – 11 917.

Smith, U. 2002. Impaired ('diabetic') insulin signaling and action occur in fat cells long before glucose intolerance — is insulin resistance initiated in the adipose tissue? Int. J. Obes. Relat. Metab. Disord. **26**: 897–904.

Storey, K.B. 1997. Metabolic regulation in mammalian hibernation: enzyme and protein adaptations. Comp. Biochem. Physiol. A. Comp. Physiol. **118**: 1115–1124.

Storey, K.B. 2001. Turning down the fires of life: metabolic regulation of hibernation and estivation. *In* Molecular mechanisms of metabolic arrest. *Edited by* K.B. Storey. BIOS Scientific Publishers, Oxford, U.K. pp. 1–21.

Storey, K.B., and Storey, J.M. 2003. Metabolic rate depression in animals: transcriptional and translational controls. Biol. Rev. In press.

Summers, S.A., and Birnbaum, M.J. 1997. A role for the serine/threonine kinase, Akt, in insulin-stimulated glucose uptake. Biochem. Soc. Trans. **25**: 981–988.

Wang, L.C.H., and Lee, T.F. 1996. Torpor and hibernation in mammals: metabolic, physiological, and biochemical adaptations. *In* Handbook of physiology, environmental physiology. *Edited by* M.J. Fregely and C.M. Blatteis. Oxford University Press, New York. pp. 507–532.

Way, J.M., Harrington, W.W., Brown, K.K., Gottschalk, W.K., Sundseth, S.S., Mansfield, T.A., Ramachandran, R.K., Willson, T.M., and Kliewer, S.A. 2001. Comprehensive messenger ribonucleic acid profiling reveals that peroxisome proliferator-activated receptor gamma activation has coordinate effects on gene expression in multiple insulin-sensitive tissues. Endocrinology, **142**: 1269–1277.