Molecular Biology of Thermoregulation
Invited Review: Molecular adaptations in mammalian hibernators: unique adaptations or generalized responses?

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van Breukelen, Frank, and Sandra L. Martin. Invited Review: Molecular adaptations in mammalian hibernators: unique adaptations or generalized responses? J Appl Physiol 92: 2640–2647, 2002; 10.1152/japplphysiol.01007.2001.—Hibernators are unique among mammals in their ability to attain, withstand, and reverse low body temperatures. Hibernators repeatedly cycle between body temperatures near zero during torpor and 37°C during euthermy. How do these mammals maintain cardiac function, cell integrity, blood fluidity, and energetic balance during their prolonged periods at low body temperature and avoid damage when they rewarm? Hibernation is often considered an example of a unique adaptation for low-temperature function in mammals. Although such adaptation is apparent at the level of whole animal physiology, it is surprisingly difficult to demonstrate clear examples of adaptations at the cellular and biochemical levels that improve function in the cold and are unique to hibernators. Instead of adaptation for improved function in the cold, the key molecular adaptations of hibernation may be to exploit the cold to depress most aspects of biochemical function and then rewarm without damage to restore optimal function of all systems. These capabilities are likely due to novel regulation of biochemical pathways shared by all mammals, including humans.

torpor; hypothermia; differential gene expression

WHEN A HUMAN IS EXPOSED TO low environmental temperatures and body temperature begins to fall, hypothermia ensues: the shivering response fails at a body temperature of 30–32°C, the heart fibrillates at 27–29°C, and ventilation ceases at 23–27°C, leading to death (reviewed in Refs. 47 and 48). However, a myriad of mammals avoid the damage associated with hypothermia by evoking controlled excursions to reduced body temperatures called torpor. In contrast to hypothermia, the reduction of body temperature in hibernators is not a pathological state (56). Deep hibernators are the masters of this adaptive hypothermia because they can maintain body tempera-
tures below 0°C for up to 3 wk (2, 26, 34). Key characteristics of torpor include a profound reduction of metabolism (up to 1/100th of basal metabolic rate), reduced heart rate, and extremely low body temperature (reviewed in Ref. 90). The physiological consequences associated with hibernation provide a natural model for the study of ischemia, muscle and bone disuse atrophy, hypothermia, ketosis, organ transplant therapy, obesity, kidney failure, and cardiac arrhythmogenesis (e.g., Refs. 18, 28, 70, 93, 95).

Ground-dwelling sciurid rodents have become the favorite model organisms for recent laboratory studies to explore the molecular bases of mammalian hibernation. In nature, these species exhibit a strict circannual rhythm of reproduction, fattening, and hibernation (for review, see Ref. 49). The cycle begins in the spring with mating, gestation, and birth. The seasons' young are
quickly weaned; these juveniles then face a particularly severe challenge to gain sufficient body mass during their first summer to survive the winter. Regardless of age, all of these animals must fatten by the end of summer, nearly doubling their body weight, to survive the winter hibernation season. The animals dig deep into the earth in the fall, seal themselves in, and remain in these burrows until the following spring, largely without eating or drinking. All is not static during this period sealed beneath the ground and snow, however. Throughout the winter months, the animals cycle between periods of torpor and arousal. The hibernation season comprises a series of bouts of torpor, lasting 1–3 wk, with core body temperatures maintained near ambient (3, 14, 37, 44). The torpor bouts are punctuated by periodic rewarmings to core body temperatures near 37°C, usually lasting <24 h, known as interbout arousals. These periodic sojourns to euthermy consume vast amounts of energy, compared with remaining at low temperature throughout the winter (reviewed in Ref. 90). Given that the purpose of torpor is to reduce energy expenditure at a time when food resources are low and the need for metabolic heat generation is high, it follows that it must be essential, either for hibernation or for survival, to return to euthermic body temperature (Ref. 56 and references therein). Rewarming occurs very rapidly, often in <2 h, whereas entrance takes much longer, usually ~1 day. Hibernation can be studied under controlled conditions in the laboratory because this rhythm is recapitulated by animals in captivity (Fig. 1) (89).

The physiology and ecology of hibernating mammals has been described in detail (for reviews, see Refs. 11, 56, 66, 91). There is also a collection of books containing reports from a series of international meetings on this topic (15, 36, 42, 45, 57). In contrast to those, this review does not attempt to provide a comprehensive treatment of the field. Rather, we address whether hibernators are uniquely adapted at the cellular and molecular level to function at low temperatures compared with other mammals, and if so, how. There is a bias by insiders and outsiders alike that biochemical pathways in hibernators must be specifically adapted to function at low temperature, compared with those pathways in nonhibernating mammals. Here, we examine the data that support this expectation. In the absence of significant evidence for such adaptations, the data suggest an alternative view that hibernators do not employ widespread adaptation to maintain function at the molecular level during the low temperatures of torpor. Rather, they exploit the low temperatures of torpor with its concomitant reduced rates of biochemical reactions as expected from temperature effects alone and then use interbout arousals to recover from the “biochemical freeze.” In this scenario, their unique adaptations largely appear in the form of resistance to damage during prolonged cold exposure, as well as during the transitions between warm and cold.

In recent years, clear evidence for depression in the cold and reactivation during interbout arousal has been obtained for several basic biochemical pathways. Cell division and migration in the intestinal epithelium are arrested during each bout of torpor and resumed during each interbout arousal (19, 52, 53). Protein translation slows dramatically during torpor but fully recovers during the interbout arousals (20, 32, 41, 50, 87, 94, 97). Protein synthesis is affected by hibernation at both the levels of initiation and elongation. Initiation is uncoupled from elongation at 18°C during
entrance into torpor (87). There may be a role for the active suppression of translation through regulatory phosphorylation of translational initiation factors (32), although this needs to be demonstrated in animals entering torpor. Elongation occurs very slowly throughout the torpor bout until preinitiated ribosomes complete their transit (41, 87); an active suppression of elongation through phosphorylation of eukaryotic elongation factor-2 seems to work in concert with temperature effects in downregulating ribosomal mean transit time threefold (20, 32).

Transcription is highly temperature sensitive and arrested to the degree expected from Q10 effects (10, 66). Our recent data from hepatic nuclear run-on assays indicate transcriptional initiation is reduced two-66). Our recent data from hepatic nuclear run-on assays indicate transcriptional initiation is reduced twofold in torpid golden-mantled ground squirrels (Spermophilus lateralis) compared with euthermic animals between bouts of torpor. In addition, transcriptional elongation rates across the temperature range experienced by hibernators suggest a virtual arrest of transcription at the low body temperatures of torpor. Complete reversal of the transcriptional arrest and even hyperactivation during the interbout arousal allows gene products to be replenished (10, 86b).

Because transcription is inhibited during torpor (10) and there is no evidence of mRNA loss during torpor (32, 68), it follows that there must also be an extension of mRNA half-life. In the absence of a mechanism to stabilize transcripts, normal turnover events would deplete pools of mRNA. Poly(A) tail lengths of liver transcripts are not reduced, and there is an association of mRNA with a poly(A) binding protein during torpor (50). Such binding may serve to prevent mRNAs from being degraded and facilitate translation when animals rewarm. During apoptosis, erythropoiesis, and spermatogenesis, transcription is arrested with concomitant accumulation of heterogeneous ectopic ribonucleoprotein (RNP)-derived structures (HERDS) (9). Similar nuclear bodies (RNPs) have been described in torpid but not in euthermic hibernators (58–62, 84), although the relevance of these RNPs to the process of hibernation remains to be elucidated. Like HERDS, these RNPs may store transcripts and various splicing factors that can be rapidly processed for expression during arousal. Similarly, cold shock domain proteins, including maskin, sequester maternal mRNAs and may prevent their translation and/or degradation during development (21, 39, 75, 80).

Taken together, these data indicate a large role for temperature in depressing gene expression at both the transcriptional and translational levels. Instead of being uniquely adapted to express genes in the cold, hibernators, in concordance with metabolic demands, depress protein synthesis. However, hibernators employ mechanisms to preserve mRNA pools that could aid in the resumption of gene expression during the interbout arousal for the replenishment of protein pools.

This same depression during torpor and reactivation during each interbout arousal is found in oxidative phosphorylation. State 3 respiration is inhibited in torpid animals yet recovers fully during each interbout arousal. This is not merely a temperature effect, because the inhibition is apparent at any assay temperature for respiration of substrate (71, 74). The inhibition does not drive the metabolic suppression or its reversal because it is not present during entrance and remains during the arousal process. State 4 respiration of succinate in liver was not affected by hibernation state (64).

What then is the molecular basis of hibernation? If hibernators have not adapted key biochemical systems to function at low temperature, how do they survive these sojourns to very low body temperatures, and why can’t we? Or can we? Clear evidence of adaptation is shown in hibernators at the level of differential gene expression at both the mRNA and protein levels, as well as for the differential control of enzymatic activity, either through phosphorylation or sequestering of enzymes.

Several mRNAs are now known to be differentially expressed during hibernation. The first example was the mRNA encoding a broad-spectrum protease inhibitor, α2-macroglobulin. Both its mRNA and protein were shown to increase seasonally during the winter (79). This protein plays a crucial role in controlling blood clotting; it is well known that clotting times of blood taken from hibernators are significantly increased (Ref. 78 and references therein). Because “improving microcirculation” in nonhibernators that have been made hypothermic (54) enhances survival, this is likely a significant adaptation for hibernation. α2-Macroglobulin is also an acute phase reactant in some animals, raising the possibility that hibernation involves a global activation of the acute phase response. Because several additional acute phase mRNAs remain unchanged during hibernation, this possibility does not appear to be the case (78). Several immediate-early gene mRNAs, including c-fos, c-jun, and junB, are differentially expressed in the brains across the hibernation cycle (68), likely reflecting the established link between neuronal activation and immediate-early gene expression. It will be particularly interesting to identify the downstream targets that are regulated by the increase of these transcription factors early in arousal. The switch from carbohydrate to fatty acid metabolism is regulated in part by differential gene expression in the heart. Elevated levels of pyruvate dehydrogenase kinase (PDK) isozyme-4 mRNA in the heart of hibernating ground squirrels likely leads to the suppression of glycolytic activity (1, 13). Pancreatic lipase, normally expressed exclusively in the pancreas, is also expressed at high levels in the heart of hibernating ground squirrels, at both the mRNA and protein levels (1). Pancreatic lipase mRNA and protein activity are also induced in white adipose tissue of hibernators, although it appears to be a distinct gene from the one induced in heart (4). The mRNA for hormone-sensitive lipase is differentially regulated in hibernators (4, 96), again consistent with an enhanced role for fatty acid metabolism during hibernation. Other reports of upregulated mRNAs in hibernators include uncoupling protein 2...
(UCP2) in white adipose tissue and UCP3 in muscle (12), NADH-ubiquinone oxidoreductase subunit 2 and ventricular myosin light chain 1 in heart (24), and glyceraldehyde-3-phosphate dehydrogenase in liver (77). Hibernators also increase expression of moesin in intestinal epithelial cells (40). Downregulation of mRNAs during hibernation has also been reported, including those for prostaglandin D2 synthase in the brain (68) and a set of plasma proteins made in liver (82, 83). However, most gene expression remains unchanged (e.g., Refs. 63, 68), perhaps not surprisingly, given the periodic resumption of “normal” function at high body temperature during each interbout arousal. Clearly, any biochemical adjustments made for function during torpor cannot compromise function during euthermy.

Specific enzymatic adaptation during hibernation has been reviewed previously (e.g., Ref. 81). Changes in enzymatic form or phosphorylation status may be important in regulating metabolism, and a variety of such changes have been noted. Interestingly, many of these changes are not consistent among tissues or species (for review, see Ref. 81). In some cases, the changes that occur could provide enhanced function at low temperature, but, in other cases, the enzymatic activity appears depressed during torpor. This incongruence may reflect tissue- or species-specific adaptation or, alternatively, may indicate a consequence of hibernation that is unrelated to the mechanism of torpor per se.

Metabolic fuel privation is one of the greater challenges faced by hibernators as they enter torpor. Glycolysis is inhibited during hibernation and daily torpor (13, 43, 67, 76, 81), and metabolism is fueled by fatty acid oxidation (33). Even when metabolism in a torpid ground squirrel is reduced to 1% of basal metabolic rate (for review, see Ref. 90), the hibernator must still be able to mobilize lipid stores for this reduced metabolism and defense of their low body temperature (14). In isolated rat hearts, high levels of fatty acids during hypothermia and rewarming cause a deficit in functional recovery (see Ref. 8). It is believed that depression of glucose oxidation by fatty acids results in greater uncoupling of glycolysis from oxidation in the tricarboxylic acid cycle with its potentially detrimental rise in H⁺ production. The buildup of acetyl-CoA reduces fatty acid utilization: acetyl-CoA carboxylase (ACC) indirectly regulates cardiac fatty acid oxidation by producing malonyl-CoA because an increase in malonyl-CoA downregulates carnitine palmitoyl transferase-1, decreasing flux of fatty acids into the mitochondrion. The production of malonyl-CoA is important in tissues like the liver where the oxidation of fatty acids competes with fatty acid synthesis. However, in the heart of a hibernator, high levels of ACC activity would inhibit the necessary fatty acid oxidation during torpor. Hibernators overcome these problems through reduced expression of the 280-kDa isoform of ACC that predominates in the heart (7) and increased expression of PDK and pancreatic lipase (1), as diagramed in Fig. 2. The depressed activity of ACC results in less malonyl-CoA (7) and subsequently allows more flux of fatty acids through the mitochondrion. Increased activity of PDK would serve to reduce glycolytic flux and limit the acid/base problems associated with uncoupled glycolysis and glucose oxidation in the presence of high levels of free fatty acids. Pancreatic lipase serves to increase fatty acid liberation at the low temperatures of torpor because, even at assay temperatures of 0°C, 34% of the maximal activity of the enzyme is retained. Analogous changes in some of these enzymes also have been reported in nonhibernators as a consequence of conditions that favor a shift from carbohydrate to fatty acid oxidation. For example, increases in PDK are seen in humans that are given an isocaloric but high-fat diet (72), and decreases in ACC

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**Fig. 2.** Model for metabolic fuel switching in the heart during torpor. Differential gene expression during winter results in reduced levels of acetyl-CoA carboxylase (ACC) and higher levels of pyruvate dehydrogenase kinase (PDK) and pancreatic lipase (PL). Increased PDK activity serves to inhibit glycolysis and reduce the accumulation of acetyl-CoA. The formation of malonyl-CoA from acetyl-CoA is further reduced by the decreased levels of ACC, and, in the absence of malonyl-CoA, carnitine palmitoyl transferase-1 delivers fatty acids liberated by PL to the mitochondrion for oxidation.
have been observed during starvation where lipid use plays a greater role in fueling metabolism (reviewed in Ref. 88). Thus many of the observed changes in gene expression that facilitate hibernation may in fact be a result of changes in metabolic fueling. In the liver, alternative mechanisms may exist, as acetyl-CoA can be converted to the ketone body β-hydroxybutyrate, which is known to increase fourfold in the blood during torpor (22).

Hibernators are unique among mammals in their ability to resist cardiac arrhythmogenesis at low body temperatures. Hearts of nonhibernator homeotherms fail when body temperatures reach ~13.0 ± 6.2°C (mean ± SD). However, in hibernators, failure occurred at 0.8 ± 2.0°C (35). Much of this resistance has been attributed to the hibernators’ ability to maintain Ca\(^{2+}\) balance (Ref. 92 and references therein). L-type Ca\(^{2+}\) channels of nonhibernators and hibernators have similar characteristics, and thus sarcoplasmic reticulum function is a more likely mechanism for the maintenance of Ca\(^{2+}\) balance (46). Sarcoplasmic reticulum activity is seasonally dependent, with the highest levels of activity during torpor (5, 6, 55), and it appears that hibernators rely more heavily on intracellular sources for activator Ca\(^{2+}\) than extracellular sources, which is in sharp contrast to the physiology of most animals (51).

Hibernation has been touted as a natural model for tolerance to ischemia by a variety of authors (e.g., Refs. 18, 31, 98). Indeed, there is increased tolerance to hypoglycemia and hypoxia in hippocampal slices from hibernating animals (30). Although blood flow may be sufficient for the reduced demands of deep torpor (see Refs. 29, 31, 73), hibernators likely face physiological ischemia during entrance into torpor. When hibernators enter torpor, heart rate diminishes before body temperature (reviewed in Ref. 65). Presumably, metabolic demand during entrance into torpor may exceed the limited supply of oxygen and nutrients. An examination of conjugated dienes, an indicator of lipid peroxidation, in the intestine of hibernators reveals a dramatic increase during the entrance phase of torpor (16). Similarly, ubiquitin conjugate concentrations increase twofold early in the torpor cycle (86a), which may reflect increased protein damage. A growing literature exists for the presence of antioxidant defenses against ischemia-reperfusion injury during torpor. Indications for antioxidant defenses include an increased concentration of plasma ascorbate to around three to fourfold over the prehibernation levels (23), increased amounts of the stress protein GRP75 and activation of nuclear factor-κB in the gut (16), a shift in glutathione redox balance to the more oxidized state (17), and possibly the presence of a tyrosyl phosphorylated protein, which may be linked to ischemic damage (69).

SUMMARY, CONCLUSIONS, AND POTENTIAL FOR HUMAN APPLICATIONS

There is no question that the physiology of hibernators is remarkable and shows profound adaptation for function under conditions that would lead to death in nonhibernators, including humans. For example, hibernators rewarm spontaneously from body temperatures that would kill an adult human even with heroic medical intervention. They do this not just once each year but numerous times throughout the winter. During torpor, their heart rates slow to 1% of the euther-mic rate, yet they recover from this without fibrillation and the heart beats normally during each interbout arousal. Somehow, cells and cell membranes maintain their integrity, presumably by maintaining ion balance. However, the evidence for molecular adaptations to improve function in specific biochemical pathways or of key proteins (e.g., Na\(^{+}\)-K\(^{+}\)-ATPase) at low temperature remains elusive. In fact, most of the key energy-consuming systems of the cell appear to simply cease function during torpor and resume activity when re-warming occurs during each inbout arousal, as detailed above.

The lack of evidence for adaptation to improve function of proteins involved in the basic processes of cell division, transcription, translation, and oxidative phosphorylation in the cold bodes well for application of biochemical strategies used during natural hibernation to achieve hypometabolism in nonhibernators, including humans. Certainly, engineering specific adaptations into each of these important systems would be a daunting task. The switch to fatty acid metabolism may be a practical solution for meeting long-term energy demands of an overwintering hibernator, although not essential for short periods of hypometabolism. If fatty acid metabolites are required to signal readiness for hibernation (e.g., Refs. 25, 27, 38), the need for this signal may be bypassed pharmacologically. Finally, the differentially expressed genes identified to date and those remaining to be identified may provide key insight into pharmacological targets important for surviving the physiological correlates of hibernation and offer targets themselves for pharmacological intervention, for example, the use of Ca\(^{2+}\) chelators to improve survival during hypothermia (48).

Superficially, the prospect of engineering a reversible hypometabolic state in humans may seem more like science fiction than science. However, evolutionary genetics based on the phylogenetic distribution of hibernation and other versions of torpor among mammalian species provides a strong argument for all mammals having the ability to resist hypothermia and achieve reversible hypometabolism. Hibernation is used by species representing all three of the deepest branches of Mammalia: it is found in placental, marsupials, and monotremes, strongly suggesting that the genetic hardware that is necessary to achieve, maintain, reverse, and survive the physiological extremes associated with hibernation is present in all mammals. Indeed, several members of primates enter daily torpor (reviewed in Ref. 34). The widespread distribution of torpor among mammals further suggests that the secret of the ability to resist hypothermia may lie with evolving a pattern of gene expression that is related to some other aspect of mammalian life. One possibility...
that comes to mind is that hibernation could involve reactivation or retention of gene expression patterns that are normally used for fetal or neonatal life. Intriguingly, moesin is normally expressed only in fetal enterocytes but is induced during hibernation (40). Furthermore, the expression of pancreatic lipase is restricted to the pancreas of adults but has been found in other tissues of neonates (85) and hibernators (1, 4). We believe that further research into the transition between the torpid and aroused states in hibernators, as well as a more focused examination of the molecular basis of differences between hypothermia and hibernation, will provide significant insight into the means of engineering reversible metabolic depression in humans.

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