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Mammalian Hibernation: Cellular and Molecular Responses to Depressed Metabolism and Low Temperature

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Carey, Hannah V., Matthew T. Andrews, and Sandra L. Martin. Mammalian Hibernation: Cellular and Molecular Responses to Depressed Metabolism and Low Temperature. *Physiol Rev* 83: 1153–1181, 2003; 10.1152/physrev.00008.2003.—Mammalian hibernators undergo a remarkable phenotypic switch that involves profound changes in physiology, morphology, and behavior in response to periods of unfavorable environmental conditions. The ability to hibernate is found throughout the class Mammalia and appears to involve differential expression of genes common to all mammals, rather than the induction of novel gene products unique to the hibernating state. The hibernation season is characterized by extended bouts of torpor, during which minimal body temperature (T_b) can fall as low as −2.9°C and metabolism can be reduced to 1% of euthermic rates. Many global biochemical and physiological processes exploit low temperatures to lower reaction rates but retain the ability to resume full activity upon rewarming. Other critical functions must continue at physiologically relevant levels during torpor and be precisely regulated even at T_b values near 0°C. Research using new tools of molecular and cellular biology is beginning to reveal how hibernators survive repeated cycles of torpor and arousal during the hibernation season. Comprehensive approaches that exploit advances in genomic and proteomic technologies are needed to further define the differentially expressed genes that distinguish the summer euthermic from winter hibernating states. Detailed understanding of hibernation from the molecular to organismal levels should enable the translation of this information to the development of a variety of hypothermic and hypometabolic strategies to improve outcomes for human and animal health.

I. INTRODUCTION

The ability to hibernate is arguably the most dramatic example of phenotypic plasticity displayed by mammals. Each year mammalian hibernators undergo a complex suite of morphological, physiological, and behavioral changes in response to seasonal periods of high-energy demand coupled with reduced energy availability in the environment. The adaptive value of the hibernating phenotype is realized by eliminating the need to maintain a

constant, high body temperature (T_b) by entering torpor. There is growing appreciation of the diversity of torpor patterns with regard to the minimum T_b values reached during torpor and the duration of torpor bouts (74, 100, 165, 256). For convenience, we have listed the common and scientific names of the species mentioned in this review in Table 1.

A typical hibernation season is characterized by extended bouts of torpor (Fig. 1) that range in duration from a few days to up to 5 wk in some species. During torpor, basal metabolic rate is reduced to 2–4% of normal rates and T_b is maintained within a few degrees above ambient temperatures (T_a). Minimal T_b values as low as −3°C have been recorded in the arctic ground squirrel (14), but they typically range from 2 to 10°C for most temperate-zone hibernators. Torpor is interrupted by periods of intense metabolic activity. During these interbout arousals (Fig. 1), T_b rises to ~36°C and is maintained for 12–24 h before reentry into torpor. Whereas most physiological functions are virtually halted during deep torpor, others continue, but at greatly reduced rates. For example, during torpor, heart rate is reduced from its normal 200–300 to 3–5 beats/min (263), respiration is reduced from 100–200 to 4–6 breaths/min and some species show long apneic periods (176), and renal function is greatly reduced or ceases altogether (263). These and other physiological parameters are restored rapidly to near-normal levels during periodic arousals. These changes, as well as the degree of hypometabolism and hypothermia experienced by mammalian hibernators, are poorly tolerated in nonhibernating species such as humans. Indeed, death due to hypothermia occurs in nonhibernating species well before the minimal T_b values of torpor have been reached.

Fat-storing hibernators eliminate food ingestion during the hibernation season and instead rely on the products of lipid hydrolysis (fatty acids and glycerol) obtained from white adipose tissue (WAT) as their primary fuel source. In contrast, food-storing hibernators such as chipmunks and hamsters used cached food that is ingested during periodic arousals and subsequently metabolized (136). Hibernation in bears is characterized by metabolic depression and extended fasting, yet their minimal T_b values during torpor remain only a few degrees below normal euthermic values (30–34°C). The seasonal increase in WAT by fat-storing hibernators can lead to a near doubling of body weight from spring emergence to fall immergence. Impressive as this fuel storage is, mammalian hibernators could not survive the 5- to 8-mo hibernation season on fatty acid metabolism alone if metabolic rate remained at active, euthermic levels. Thus the profound metabolic depression characteristic of the torpid state significantly reduces energy costs and is therefore a critical component of the hibernating phenotype.

The fact that some seasonal hibernators can undergo such profound physiological remodeling even in the absence of external input from environmental cues provides strong evidence that the ability to hibernate is driven by a molecular genetic mechanism rather than being an acute physiological response to periodic food scarcity and/or low ambient temperatures. Since the work of Dubois (81) and others in the late 1800s, scientists have studied and speculated on the mechanisms of mammalian hiberna-

TABLE 1. *Species used for hibernation and torpor studies that are mentioned specifically in this review*

Species Name	Common Name	Major Group	Figure 2
Tachyglossus aculeatus	Short-beaked echidna	Monotreme	a
Elephantulus myurus	Elephant shrew	Macroscelidea	b
Elephantulus rozeti	Elephant shrew	Macroscelidea	b
Spermophilus parryii	Arctic ground squirrel	Rodentia (sciurid)	c
Spermophilus citellus	European ground squirrel	Rodentia (sciurid)	c
Spermophilus lateralis	Golden-mantled ground squirrel	Rodentia (sciurid)	c
Spermophilus tridecemlineatus	Thirteen-lined ground squirrel	Rodentia (sciurid)	c
Spermophilus richardsonii	Richardson's ground squirrel	Rodentia (sciurid)	c
Spermophilus columbianus	Columbian ground squirrel	Rodentia (sciurid)	c
Marmota marmota	Alpine marmot	Rodentia (sciurid)	c
Marmota flaviventris	Yellow-bellied marmot	Rodentia (sciurid)	c
Marmota monax	Woodchuck	Rodentia (sciurid)	c
Cynomys ludovicianus	Black-tailed prairie dog	Rodentia (sciurid)	c
Cynomys leucurus	White-tailed prairie dog	Rodentia (sciurid)	c
Tamias sibericus	Siberian chipmunk	Rodentia (sciurid)	c
Jaculus orientalis	Greater Egyptian jerboa	Rodentia	c
Glis glis (Myoxus glis)	Edible dormouse (fat dormouse)	Rodentia	c
Mesocricetus auratus	Syrian hamster	Rodentia	c
Mesocricetus brandti	Turkish hamster	Rodentia	c
Cheirogaleus medius	Fat-tailed dwarf lemur	Primate	d
Ursus americanus	Black bear	Carnivora	e
Myotis lucifugus	Little brown bat	Chiroptera	f
Erinaceus europaeus	European hedgehog	Eulipotyphia	g

Letters in last column indicate placement of these species in Figure 2.

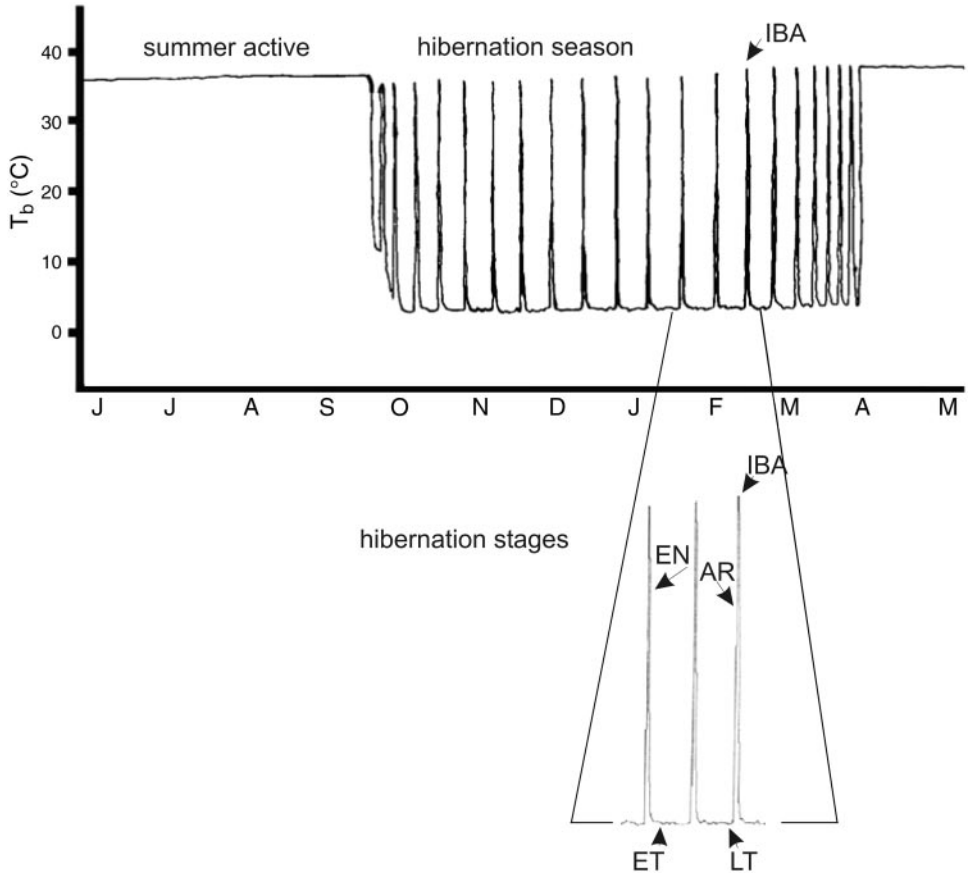

FIG. 1. Body temperature (T_b) as a function of time in a golden-mantled ground squirrel. *Top:* typical T_b pattern for a single animal during 1 yr. Time is plotted on the *x*-axis, from June through May. Body temperature (0–40°C) is plotted on the *y*-axis. IBA, interbout aroused. *Bottom:* enlargement of region encompassing three torpor-arousal cyles. EN, entrance (duration ~12 h in golden-mantled ground squirrels); ET, early torpor (within 48 h of entering a torpor bout); LT, late torpor (>7 days into a 10- to 14-day torpor bout); AR, arousing (duration ~2 h); IBA, interbout aroused (duration ~20 h). [Modified from Epperson and Martin (84).]

tion. Although there is now a considerable amount of information detailing various aspects of the morphological, physiological, and biochemical changes that are associated with hibernation, the cellular and molecular bases of mammalian hibernation are still poorly understood. This review focuses on the latter topic and as such, we do not include information on organ and systemic physiology of hibernation unless it is pertinent to illustrate hibernation-specific effects at the cellular and molecular levels. Parallels can be drawn between mammalian hibernators and other nonmammalian organisms that undergo extended periods of metabolic depression as part of their normal life cycles. Several reviews and conference proceedings are available for aspects of hibernation biology (28, 45, 98, 100, 117, 120, 153, 167, 169, 186, 247), for mammals that undergo daily torpor and its comparison to hibernation (100, 165, 256), and for nonmammalian metabolic depression (26, 109, 112) that are not detailed here.

II. EVOLUTIONARY PHYLOGENY OF MAMMALIAN HIBERNATION AND TORPOR PATTERNS

Hibernating species are widely distributed within the class Mammalia; all three of the deepest branches of this group, i.e., placentals, marsupials, and monotremes, contain hibernators (Fig. 2). In each of these lineages, species or groups of species that hibernate are interspersed with species that do not. This patchwork distribution pattern of hibernators upon the phylogeny of mammals, taken together with the fact that some birds also hibernate (100), is most parsimonious with the conclusion that hibernation is a retained ancestral trait in modern mammals. Whether hibernation is ancestral or a newly developed trait, the widespread distribution of mammalian species that hibernate suggests that the genes required to specify the hibernating phenotype are common among the genomes of all mammals (223).

Given a common genotype that is capable of generating the hibernating phenotype, why is it that some species hibernate and some do not? This question would be best answered by careful studies of a pair of closely related species, one that is able to hibernate, and one that cannot. However, such pairs are not readily identified; typically, all of the close relatives of one hibernating species are themselves hibernators (as in the ground-dwelling sciurid rodents) or at least capable of reversible metabolic depression in the form of daily torpor. There are some examples of relatively closely related species within the same genus where one is a seasonal, deep hibernator (white-tailed prairie dog) and the other is a

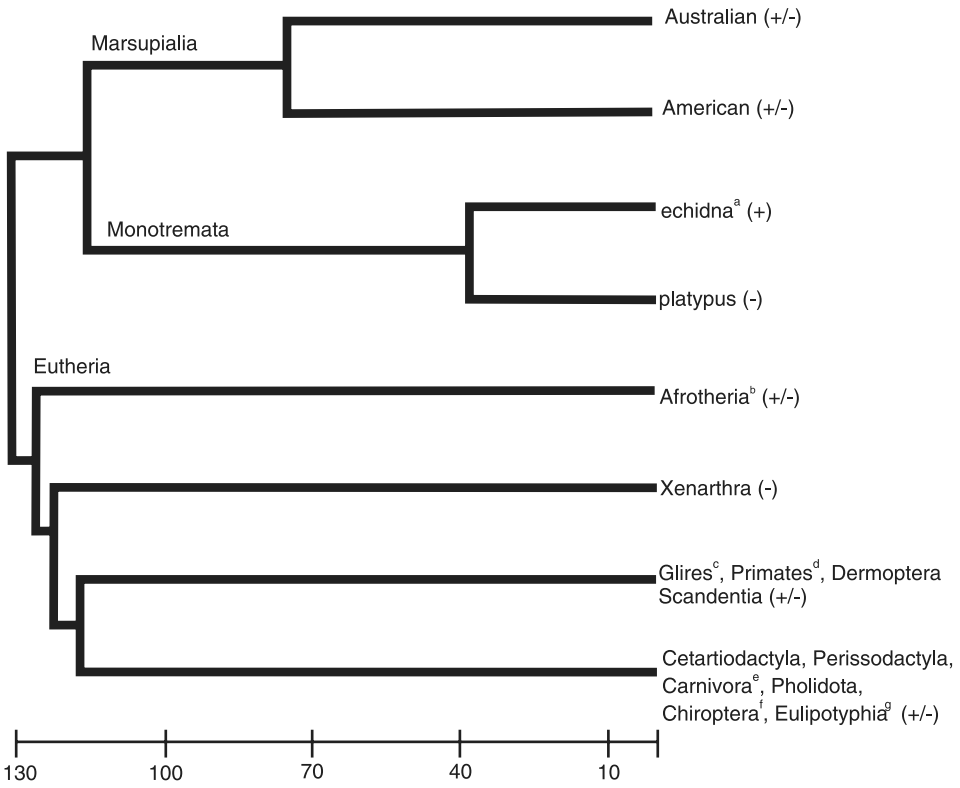

FIG. 2. Dendrogram depicting relationships of mammalian species that use hibernation or torpor. Time scale is in millions of years. Hibernating species are found in all of the deepest branches of mammals. This distribution suggests and is consistent with the hypothesis that all mammals share the genes required for hibernation. The tree structure is based on data in Refs. 139, 140 for marsupial, monotreme, and eutherian divergence and in Ref. 185 for the branches of eutherian mammals. Examples of species that use hibernation or torpor in each of these lineages are as follows: [a]echidna; [b]elephant shrews (165); [c]jerboas, hamsters, dormice, and sciurids, including marmots, ground squirrels, and chipmunks; [d]lemur (74); [e]bears; [f]bats; and [g]hedgehogs. Scientific names for these groups are indicated in Table 1.

facultative hibernator (black-tailed prairie dog) (113). In fact, the use of hibernation and torpor by mammals in the wild, and thus the ability to hibernate, is probably much more widespread than is presently appreciated (97).

III. CHANGES IN GENE EXPRESSION

In biology, a common genotype typically gives rise to a novel phenotype via altered patterns of gene expression. Gene expression can be controlled at multiple levels, including mRNA transcription, processing and stability, as well as protein translation, processing, stability, and posttranslational modifications or localization. Although the gene expression pattern that creates the hibernating phenotype remains to be elucidated in detail, sufficient progress has been made to indicate that hibernation is no exception to this paradigm.

Hibernation's unique characteristics as a phenotype likely constrain the types of molecular changes that are employed. Seasonal changes prepare the animal for hibernation; one example is the storage of enormous reserves of energy in the form of WAT in late summer and early fall. These seasonal changes are likely achieved by altering steady-state levels of proteins that serve a specific function through increasing or decreasing the abundance of the corresponding mRNA and protein. In contrast, each cycle of torpor and arousal during the hibernation season may be regulated less by changing the amounts of gene product via altered transcription, translation, or stability and more by employing rapidly reversible molecular switches such as phosphorylation to control protein activity (226). Because the hibernating mammal arouses periodically from torpor throughout the hibernation season (Fig. 1), the ability to function at euthermic temperatures must be maintained continuously. At the molecular level, this predicts that the steady-state levels of mRNA and protein expressed from the majority of genes will remain relatively constant between the active and hibernating seasons. A relatively small number of genes that perform crucial functions for hibernation will be upregulated during winter, whereas other gene products that are not essential during hibernation may be downregulated. An alternative explanation for downregulation of certain gene products during hibernation that could be significant is that the activity of certain gene products may inhibit hibernation (for example, by promoting arousal); downregulation of such gene products would be required before hibernation could ensue.

Several mRNAs are now known to be differentially expressed during hibernation. The first example of a seasonally upregulated gene at the mRNA level in hibernators was α_2-macroglobulin, which encodes a broad-spectrum protease inhibitor that is produced in the liver then secreted into the plasma (223). Both its mRNA and protein were shown to increase during winter in ground squirrels. This protein plays a pivotal role in controlling blood clot-

ting; it is well-known that clotting times of blood taken from hibernators are significantly increased (222). Because improving microcirculation in hypothermic rats (163) and preventing or reversing blood clotting in cardiac arrest patients (31) enhances survival, reduced clotting is likely a significant adaptation for hibernation. α_2-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 does not appear to be the case (222). In liver, the genes encoding thyroxine-binding globulin, apolipoprotein A1, and cathepsin H are also all upregulated at the mRNA level during the hibernation season (84).

The mRNAs for the immediate-early genes, c-*fos*, c-*jun*, and junB are differentially expressed in ground squirrel brain across the hibernation cycle (192). This likely reflects the established link between neuronal activation and immediate-early gene expression (181). It will be of particular interest to identify the downstream targets that are regulated by the increased expression of these transcription factors early in arousal. Increased amounts as well as an increased field of expression of neuropeptide Y mRNA were observed by in situ hybridization in the arcuate nucleus of the hypothalamus in hibernating jerboas (83).

The switch from carbohydrate to fatty acid metabolism is regulated by differential gene expression at multiple levels, including altering the steady-state levels of key proteins and the mRNAs encoding them. The general pattern is to suppress carbohydrate metabolism and increase fatty acid metabolism. Elevated levels of pyruvate dehydrogenase kinase isoenzyme 4 (PDK4) mRNA and protein in hibernating ground squirrels suppress glycolytic activity (4, 35). Pancreatic triacylglycerol lipase (PTL), which hydrolyzes ester linkages in dietary triacylglycerols and is normally expressed exclusively in pancreas, is also expressed at high levels in the heart of hibernating ground squirrels, at both the mRNA and protein levels (4). Glyceraldehyde-3-phosphate dehydrogenase is downregulated at the mRNA, protein, and activity levels in skeletal muscle of hibernating jerboas (218, 219), as is acetyl CoA carboxylase in the hearts of hibernating ground squirrels (20). PTL mRNA and protein activity are induced in white adipose tissue of hibernating ground squirrels, although the mRNA expressed in WAT is distinct from the one induced in heart (17). The mRNA for hormone-sensitive lipase (HSL), which is necessary for release of fatty acids from WAT, is also differentially regulated in hibernating marmots (255) and ground squirrels (17), consistent with the enhanced role of fatty acid metabolism during the hibernation season.

Other reports of upregulated mRNAs in hibernating ground squirrels include uncoupling protein 2 (UCP2) in WAT and UCP3 in skeletal muscle (29); heart-type fatty acid binding protein (FABP) in brown adipose tissue (BAT), skeletal muscle, and heart (125); adipose type FABP in BAT and heart (125); cytochrome-*c* oxidase subunit 1 and ATP synthase 6/8 in kidney, BAT, and heart (127); and NADH-ubiquinone oxidoreductase subunit 2 in heart, skeletal muscle, and liver; and ventricular myosin light chain 1 in heart and skeletal muscle (85).

Downregulated mRNAs during hibernation also have been reported, including those for prostaglandin D_2 synthase in the brain of ground squirrels (192); glyceraldehyde-3-phosphate dehydrogenase in the muscle of jerboas (219); and liver-expressed HP-22, -25, -27, and -55 which form a protein complex in chipmunk plasma that is also decreased in hibernation (152, 231, 232). Recent work on the plasma proteins HP-25 (150, 151), HP-20 (195), and HP-27 (196) has focused on the liver-specific regulation of their expression at the transcriptional level.

Many of the mRNAs that have been reported to change either seasonally or as a function of hibernation stage have been examined in a small number of individuals by blotting methods. If the changes in the steady-state levels of these various mRNAs are truly significant for hibernation, it will be necessary to demonstrate consistent patterns in large numbers of individuals and in different species, as well as corresponding changes in the amount and/or activity of the encoded proteins. If there is discordance among findings obtained from different species of hibernators, as is often found with the older literature in this field, this may be due to utilization of different gene products that lead to the same final result. Another source of discordance in the hibernation literature stems from the use of animals treated differently, or when the stages of torpor are not considered independently. In the case of seasonal hibernators, there is questionable biological relevance of a winter (fat) animal kept in warm conditions or with light-dark cycles, or a summer animal (thin) kept in conditions of constant cold, and results obtained from studies that utilize these types of "controls" should be considered carefully. Ideal controls for hibernation studies occur thanks to the nature of the annual cycle; summer active animals should be compared with winter active (i.e., interbout aroused) and torpid hibernators. This experimental design provides controls for both external and internal effects (e.g., body temperature, environmental temperature, feeding status) without stressing the animals. Furthermore, studies that attempt to characterize the changes associated with hibernation must distinguish between torpid animals and those in other states within the hibernation season including entrance, arousal, and interbout euthermic animals (Fig. 1). The biochemical properties of these distinct physiological states are proving to be profoundly different.

At the protein level, myoglobin appears to increase in skeletal muscle as animals prepare for, and undergo, hi-

bernation. This increase may facilitate shivering thermogenesis during torpor and arousal (203). Immunoreactivity for the gap junction protein connexin43 is elevated in cardiac myocytes of hibernating hamsters and reverses to euthermic control values within 2 h of arousal to euthermy (211). It was suggested that an increase in gap junctions may prevent ventricular fibrillation at the low T_b values of torpor. However, because connexin43 immunoreactivity is elevated in cold-acclimated control hamsters that did not hibernate (211), this change is not strictly associated with the hibernating phenotype. A particularly intriguing pattern was reported for moesin in intestinal epithelial cells. The protein is detected readily during torpor in hibernating ground squirrels by both Western blotting and immunocytochemistry, yet not in summer animals or in the interbout aroused animals during hibernation (104). It will be interesting to determine whether this result reflects biosynthesis of moesin during torpor or the entrance into the torpid state. Production of moesin during deep torpor would be surprising given the generalized depressions of transcription and translation that characterize torpor (see below).

Significantly, the steady-state levels of mRNAs and proteins corresponding to the vast majority of genes appear to remain constant throughout the seasons and stages of hibernation (53, 84, 192, 222), consistent with resumption of normal cellular function during the numerous euthermic periods that punctuate the hibernation season. The results of several studies provide indirect evidence for prolonged mRNA half-life during torpor; no change was observed in the steady-state levels of several specific mRNAs as assessed by Northern blotting (192) or quantitative RT-PCR (84), or in the overall length of mRNA poly(A) tails (149) across torpor bouts. The prolonged mRNA half-life for 12 mRNAs expressed in liver and assessed by quantitative RT-PCR during torpor is substantially greater than predicted from Q_{10} effects alone (84).

The fact that at least one species of mammalian hibernator, the arctic ground squirrel, maintains subzero T_b values during deep torpor (14) raises the possibility that specialized antifreeze proteins similar to those found in Antarctic fishes might be expressed during hibernation that would prevent freezing of body fluids at T_b values below 0°C. To date, there is no evidence for antifreeze activity such as thermal hysteresis in blood from supercooled arctic ground squirrels (14).

Another critical level of regulation occurs at the level of posttranslational modification, which may control the activity of a protein directly or indirectly by changing its location or association with other proteins. The known roles of these types of changes in hibernation are discussed in more detail elsewhere in this review, particularly in the sections about protein translation and carbohydrate metabolism, and have been reviewed previously (226). Glutamate dehydrogenase isolated from torpid and euthermic Richardson's ground squirrels had distinct biochemical properties, yet behaved indistinguishably by SDS-PAGE (234). Control of a protein's activity by reversible phosphorylation is particularly well suited to play a crucial role as animals transition between torpor and interbout arousal because of the rapidity of the changes and the reduced rates of transcription and translation. Na^+-K^+-ATPase activity decreases by 60% during hibernation in muscle extracts prepared from golden-mantled ground squirrels. Similar levels of suppression can be achieved by in vitro stimulation of protein kinase activity. Conversely, addition of protein phosphatases to hibernating muscle extract increased Na^+-K^+-ATPase activity, consistent with a regulated, reversible suppression of this significant energy-consuming protein during torpor (168). An antibody specific for phosphotyrosine reacted with an unidentified protein of unknown function in plasma membranes extracted from torpid but not active ground squirrel brain. The same phosphoprotein, pp98, was not detected in squirrels as early as 1 h after arousal; thus either the modification or the protein is specific to the torpid state (193). Clearly, many critical regulatory events occur in hibernators via phosphorylation or other posttranslational modifications of proteins, yet most remain to be understood.

IV. METABOLIC ADAPTATIONS

A. Initiation and Reversal of Torpor

The molecular basis for the initiation and reversal of torpor during mammalian hibernation remains unknown. Although hibernating animals retain the ability to sense and defend T_b, as they enter torpor the hypothalamic setpoint for T_b regulation is gradually lowered (119) until T_a reaches the lower critical temperature (e.g., close to 0°C for hibernating arctic ground squirrels, Ref. 34). Metabolic rate drops precipitously before a significant drop in T_b during entrance (118, 198), indicating that reduced rates of reactions due to temperature effects alone are not driving entrance into torpor. It is tempting to speculate that the secret to understanding entrance into torpor is as "simple" as understanding the mechanism for lowering the T_b setpoint (119).

Although many hypotheses have been proposed for the signal(s) that initiate the periodic arousals from torpor, there are no good data to explain this process, particularly at the molecular level. Clearly, many physiological processes benefit from the brief euthermic periods during the hibernation season, but it is unknown whether any serve as primary stimuli for arousals. Except for dietary modifications (i.e., changes in lipid composition) or changes in T_a, there are few manipulations that have

been shown to significantly alter the duration of torpor bouts. Mechanistically, it is reasonable to assume that a change in the T_b setpoint near the end of a torpor bout plays a role in initiating the thermoregulatory responses that lead to increased metabolic rate and a rapid return of T_b to euthermic levels. Although no molecules have been confirmed as essential for either the entrance or arousal from torpor, several neurotransmitters that influence mammalian thermoregulation including serotonin, histamine, and opioids have been proposed to play important roles in the central nervous system (CNS) regulation of torpor patterns (110, 154, 212).

There is great interest in identifying a specific factor(s) that induces torpor in mammals. Although more than three decades have passed since the initial report of a blood-borne agent that appeared to induce hibernation (76), neither purification nor identification of a bona fide hibernation induction trigger, or HIT, has been achieved. The original bioassay for HIT was the induction of hibernation in summer-active 13-lined ground squirrels by intravenous injection of an albumin-containing plasma fraction from torpid hibernators such as woodchucks and black bears. At least part of the problem in purifying the HIT appears to stem from the use of this difficult and unreliable bioassay to measure the hibernation-inducing activity (167, 245, 248). A series of investigations have linked the "HIT" to a δ-opioid activity (132), although at this time it is not justified to consider this activity a trigger that induces hibernation. There is, however, considerable evidence to suggest that hibernation is accompanied by changes in the brain opioid system (19, 70).

Observations from several laboratories on sleep characteristics in torpid and euthermic ground squirrels during the hibernation season led to the intriguing hypothesis that the restorative function of sleep may be compromised by the low T_b values of torpor, resulting in a gradual accumulation of slow wave activity (SWA) debt during torpor bouts. The need to restore a sleep debt was therefore suggested as a trigger for the initiation of periodic arousals from torpor (72, 239). Subsequent studies have not supported the idea that arousals are triggered by SWA debt or that arousal episodes are necessary for sleep homeostasis in hibernators (160, 161, 228, 229). However, it is still possible that interbout euthermic periods during the hibernation season may restore or repair some other critical neurological process that is altered after extended periods at low T_b such as brain energy stores or synaptogenesis (161).

B. Shifts in Fuel Utilization

Several factors point to a reduction in carbohydrate oxidation and a reliance on the combustion of fat during hibernation. There are substantial increases in body fat preceding the hibernation season with a concomitant loss of much of this fat during the hibernating period (24, 144). The respiratory quotient (RQ), i.e., the ratio of the volume of carbon dioxide produced to the volume of oxygen consumed, indicates which substrate is being used for energy production; an RQ of 1.0 suggests carbohydrates as the source of energy, whereas an RQ of 0.7 suggests lipids. Hibernators in torpor typically display RQ values near 0.7, indicating that fatty acids are their main source of energy (220). During periodic and terminal arousals from torpor, RQ values between 0.8 and 1.0 have been reported (reviewed in Refs. 220, 246). Although part of this rise has been attributed to hyperventilation, the other part has been linked to oxidation of carbohydrate stores found in liver and muscle. In torpid arctic ground squirrels, RQ rises to values greater than 0.85 when T_a is increased from 8 to 20°C or decreased from 4 to −16°C (34). Thus lipids are the sole source of fuel for these animals only during torpor, within the narrow temperature range of 4–8°C.

Direct evidence for the switch from carbohydrate- to fat-based metabolism comes from studies that measured glycolytic rates (40) and glycolytic enzyme activity (32), the metabolic fates of radiolabeled glucose as a carbon source (233), lipogenic and lipolytic enzyme activity (17, 88), and the capacity for gluconeogenesis using various metabolic substrates (39, 95, 107, 224). These and other studies demonstrate that although the hibernator may have a greater capacity for anaerobic glycolysis than its nonhibernating counterpart (40), less glucose is being oxidized during hibernation (233). Furthermore, a decrease in pyruvate dehydrogenase activity in heart and kidney (32) is consistent with the observation that glycolytic intermediates are not entering the tricarboxylic acid cycle during hibernation or during the beginning of arousals from the hibernating state (95, 233). Glycerol, which is liberated when triacylglycerols are hydrolyzed in WAT, is used as a substrate by the liver and kidney to generate glucose via gluconeogenesis (39, 107, 224, 262). This process also facilitates replenishment of glycogen reserves that are partially depleted during torpor or arousal (95).

A search for genes that are upregulated in hibernating 13-lined ground squirrels resulted in the identification of two proteins whose differential expression offers a possible mechanism for genetic control of fuel selection during hibernation (4). Both PDK4 and PTL mRNAs increase during hibernation in several ground squirrel tissues (4, 17, 35). Levels of PDK4 increase during hibernation in heart, skeletal muscle, and WAT of 13-lined ground squirrels and likely convert pyruvate dehydrogenase (PDH) to its inactive form by phosphorylation. This modification of PDH impedes carbohydrate catabolism by blocking the conversion of pyruvate to acetyl CoA.

PTL, on the other hand, hydrolyzes triacylglycerols to liberate fatty acids for β-oxidation. In 13-lined ground

squirrels PTL exhibits >30% maximal activity at 0°C (4), a T_b attained in some hibernators during winter (100). Both PTL and PDK4 work coordinately to support the switch from carbohydrate to lipid catabolism. In WAT, both HSL activity (88) and PTL activity (17) offer a steady supply of nonesterified fatty acids that can be reesterified as triacylglycerols in other tissues. In the heart, these triacylglycerols are stored as mitochondria-associated lipid droplets and provide the foundation for ensuing ATP generation (37).

An increase in insulin concentration has been shown to reduce the levels of both PDK4 protein and mRNA in rat heart and skeletal muscle (258, 260). Levels of serum insulin increase as ground squirrels fatten before hibernation and then decline to their lowest measured levels in December-January hibernators. These low levels persist during interbout arousals (35). This pattern of serum insulin levels resembles that seen in yellow-bellied marmots (236). Despite reduced serum insulin levels in hibernating 13-lined ground squirrels, insulin mRNA in the pancreas is highest in animals hibernating from December through March (35). This observation is analogous to elevated pancreatic insulin concentration in hibernating little brown bats; during hibernation, the level of insulin in bat pancreas steadily increases, reaching its maximal level just before spring arousal (18). The increase in pancreatic insulin concentration from early to late in the hibernation season may provide a rapidly releasable pool available for immediate secretion after spring emergence (18). An obvious benefit for the hibernator is facilitation of rapid glucose uptake after feeding resumes in the spring.

Whereas insulin acts as an inhibitor of PDK4 expression, a member of the ligand-dependent peroxisome proliferator-activated receptor (PPAR) family is known to activate the PDK4 gene. PDK4 expression is induced in skeletal muscle of rats and hearts of mice by a mechanism involving PPARα (258, 259). Natural PPAR-activating ligands include long-chain free fatty acids, such as linoleic acid, linolenic acid, and arachidonic acid (148). Several studies suggest that essential fatty acids including linoleic acid are necessary for normal hibernation patterns (87, 90, 99, 113, 124). Thus the timing of increased PDK4 expression in 13-lined ground squirrels could result from fatty acids that are obtained from the diet preceding hibernation (86). This is supported by the observation that pharmacological activation of PDK4 expression can be achieved by administration of the fatty acids palmitate and oleate (133), and elevated levels of fatty acids are observed in serum before and during hibernation (96). Furthermore, starvation (257, 258, 259, 260) and diabetes (260), two conditions that share some features with the hibernating state, also stimulate PDK4 expression.

Given four known PDK isoenzymes in mammals (208), why is PDK4 the gene that is upregulated in heart, skeletal muscle, and WAT during hibernation (35)? Part of the answer possibly lies in the differing sensitivities of the PDKs to dichloroacetate, a pyruvate analog and synthetic inhibitor of PDK activity (reviewed in Ref. 27). Of the four isoenzymes, PDK4 is the least sensitive to inhibition by dichloroacetate (27). Thus PDK4 activity is less likely to be inhibited if there is an accumulation of pyruvate during hibernation.

PPARα has also been shown to activate genes involved in lipid metabolism (214). Of potential importance to hibernation is the role of PPARα in coordinating PDK4 gene expression with the expression of genes responsible for extracellular and intracellular lipid transport and mitochondrial β-oxidation of fatty acids (reviewed in Ref. 221). Activation of PPARα could therefore provide a mechanism linking the inhibition of carbohydrate oxidation with increased fat catabolism, thus accounting for the switch in fuel selection during hibernation. PPARα activity has also been associated with expression of UCP3 (33), whose mRNA is elevated in skeletal muscle during hibernation (29). A model depicting pathways involved in the molecular regulation of carbon utilization before and during the hibernation season is shown in Figure 3.

V. THERMAL ADAPTATIONS

Torpor involves a global suppression of physiological processes; there are essentially no processes that continue at normal, euthermic rates during the low T_b values of deep torpor. Yet, certain critical functions must continue at physiologically relevant levels during torpor and be precisely regulated even at T_b values close to or below 0°C. In this section we first describe some of the processes whose functions are severely depressed at the T_b of torpor but resume normal or supranormal function during interbout arousals; these processes are physiologically less critical for survival during torpor but necessary for overall survival during and after the hibernation season. We then highlight those processes that are suppressed, but continue during torpor and are therefore likely to be highly physiologically relevant for survival during torpor. Notable among these are CNS, cardiac and respiratory functions, and lipolysis in adipose tissue.

A. Processes Suppressed at Low Temperature and Reversed During Arousal

One hypothesis that has prevailed in the hibernation literature is that relative to other mammals, hibernators are adapted at multiple levels for improved biochemical function in the cold. Despite indisputable evidence for improved physiological function of many systems at low T_b in the cold (e.g., the heart does not fibrillate), there is little evidence to support an underlying biochemical ad-

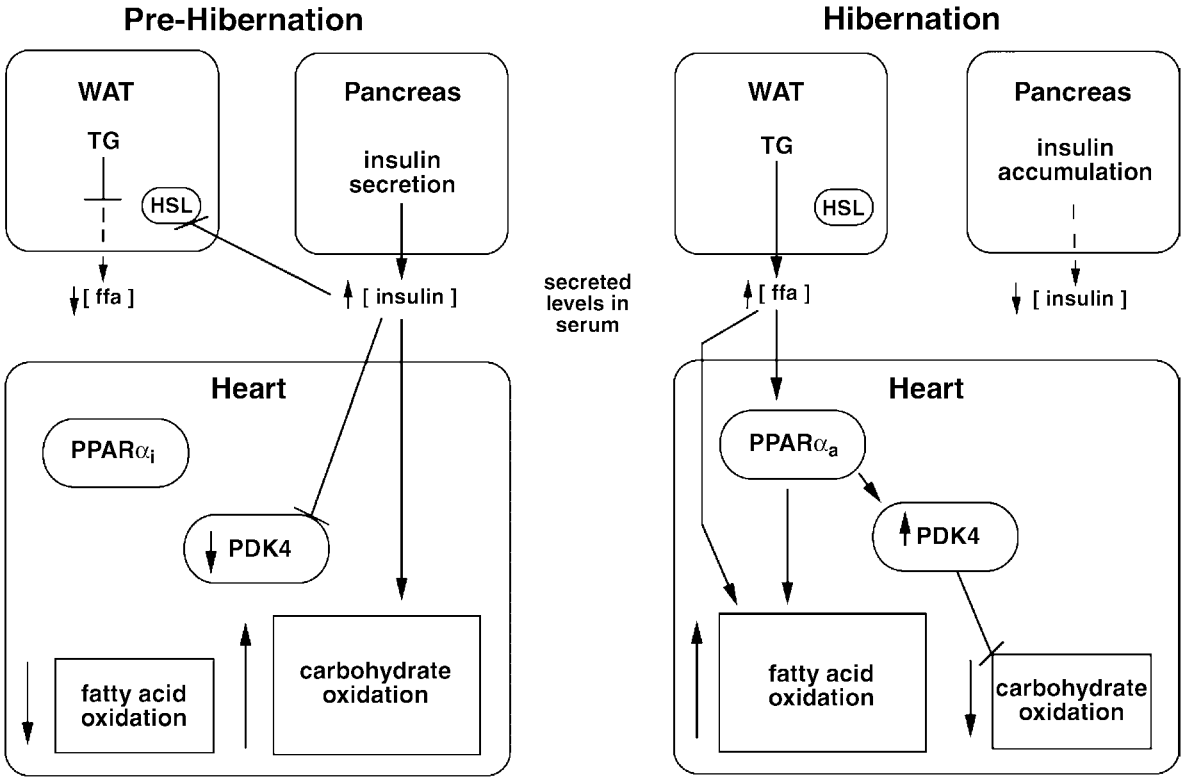

FIG. 3. Model showing regulation of the switch from carbohydrate to fatty acids as the primary source of fuel during hibernation. Shown are effects of serum levels of insulin secreted from the pancreas and free fatty acids secreted from white adipose tissue (WAT) on pyruvate dehydrogenase kinase isoenzyme 4 (PDK4) gene expression, carbohydrate oxidation, and fatty acid oxidation in heart of animals in prehibernation (September-October) and hibernation (December-January). Lines with arrowheads indicate upregulation or activation, and lines with blunt ends indicate downregulation or inhibition. Solid lines show the active or predominant mode of regulation, and dashed lines show minor pathways. Short vertical arrows pointing up or down indicate an increase or decrease, respectively, in concentration or activity. ffa, Free fatty acids; HSL, hormone-sensitive lipase; PPARα, peroxisome proliferator activated receptor α; PPARα_i, inactive receptor; PPARα_a, active receptor; TG, triacylglycerols.

aptation (243). Significantly, a number of global biochemical processes appear to exploit the cold to lower reaction rates, but retain the ability to resume full activity upon each rewarming. This concept appears to apply to functions such as transcription, translation, cell proliferation, mitochondrial respiration, and other functions including digestive and renal physiology and the immune response. Other features also exhibit change during torpor and reversal upon arousal including some aspects of cell morphology and membrane structure and function. To date there is little evidence that restoration of any of these processes dictates the timing of interbout arousals.

1. Transcription

RNA synthesis is an energetically expensive process. The global activity of the transcriptional apparatus during torpor was first assayed by examining the conversion of injected radiolabeled nucleotide precursor into acid-insoluble material in animals at various stages of the annual cycle. By this approach, transcription slowed significantly with UTP incorporation dropping to 11% of euthermic values during torpor compared with aroused hibernators (25). More recently, nuclear run-off assays were used to examine in more detail the aspects of transcription that are affected. Nuclear run-off assays were performed under controlled conditions in vitro, using liver cell nuclei isolated from animals in different stages of the annual hibernation cycle (244). These assays also rely on conversion of radiolabled ribonucleotide into acid-insoluable material (RNA transcripts), but eliminate any questions regarding differences between the delivery of isotope to the site of transcription, or specific activity of the isotope within the cells. Initiation of transcription does not occur to any appreciable extent once nuclei are isolated; therefore, the incorporation measured in the assay is into transcripts that were already initiated in the animal. This assay can also be used to measure the effect of the various temperatures experienced by hibernators on the rates of transcriptional elongation by simply incubating the nuclei at temperatures ranging from 0 to 37°C.

Results of such studies demonstrated that global transcription is depressed in torpid golden-mantled ground squirrels due to the combination of a twofold reduction in initiation during torpor and the intrinsic sensitivity of elongation to temperature (rates at 5°C ≤10% of 25°C, Ref. 244). The combined effect on transcriptional initiation and elongation is so severe that mRNA synthesis likely ceases during torpor in golden-mantled ground squirrels (244). Because the temperature reductions in the rate of transcriptional elongation were identical among nuclei isolated from rats and summer-active, torpid, and interbout-aroused ground squirrels, it does not appear that the basal transcription machinery is uniquely modified or adapted for improved function in the cold during hibernation or in a hibernating species compared with a nonhibernator (244). The observed temperature sensitivity of all samples fell within the boundaries provided by a Q_{10} of 2–3, suggesting that reduced transcriptional rates during torpor are due to simple temperature effects on reaction rates and do not require active mechanisms to control suppression (244).

2. Translation

Translation of mRNA into protein is even more expensive energetically than transcription. Consistent with a need to reduce energy consumption during torpor, several studies have demonstrated reduced protein synthesis in hibernators. As with transcription, the earliest studies measured conversion of injected radiolabeled amino acids into acid-insoluble material in animals in different stages of the hibernation cycle (264). The results revealed significant depression of protein synthesis in torpid ground squirrels compared with other states. In addition, protein synthesis in heart, liver, spleen, pancreas, and kidney during the interbout euthermic period was elevated relative to euthermic summer animals (264). The small amount of residual activity measured in this type of assay during torpor could not be detected in brain slices or as specific polypeptides when radiolabeled proteins were extracted from brain and subsequently displayed by SDS-PAGE (93).

Results of several studies demonstrated significant loss of polyribosomes in brain, liver, and kidney extracts from torpid hibernators (93, 126, 149, 242), further supporting the results from in vivo labeling that translation is depressed during torpor. An exception to this general observation may be BAT where polyribosomes appear to remain intact and protein synthesis may continue during torpor (126). Comparisons of the distribution of actin mRNA in polysome gradients prepared from liver extracts taken from different stages of hibernation revealed the highly dynamic nature of protein synthesis across a torpor bout (242). Interbout arousal is characterized by association of all of the actin mRNA with heavy polyribosomes, indicating maximal levels of protein synthesis, which is also found in summer-active ground squirrels (242). As animals enter torpor, this pattern is maintained at temperatures above 18°C (242). Therefore, if there are temperature effects that reduce translation as hibernators cool, either they do not occur above 18°C, or they affect initiation and elongation rates synchronously. Below 18°C, however, actin mRNA begins to redistribute off of polyribosomes, suggesting that initiation is relatively slowed or blocked at the low temperatures of torpor, but elongation continues (242). This result is predicted if no further initiation occurs below 18°C. In contrast, elongation must continue (albeit slowly due to Q_{10} effects) even at the low body temperatures of torpor because loss of actin mRNA from polyribosomes between early and late torpor is apparent; late in torpor, all of the actin mRNA is free and no longer associated with polyribosomes. Intriguingly, early in arousal, as body temperature rises but before reaching 18°C, actin mRNA begins to redistribute onto polyribosomes, indicating that the block to initiation is reversed and also implying it is not strictly due to simple temperature effects. The fully active pattern, however, is not restored until the animal's body temperature reaches 18°C (242).

Evidence for translational suppression is also provided by examining brain extracts for their ability to support translation in vitro. Extracts from hibernators are significantly less active in this assay than those from active animals. Furthermore, the suppression can be overcome by incubating polyribosomes isolated from hibernators in extracts prepared from active animals, indicating that active translation factors are limiting in the hibernators (93). Both initiation and elongation appear to be affected. Phosphorylation of the initiation factor eIF2α is increased in hibernators compared with active animals and may contribute to the suppressed initiation of protein synthesis during hibernation (93). This increased phosphorylation during hibernation is correlated with disrupted interactions between growth arrest and DNA damage-inducible protein (GADD34) and both type 1 protein serine/threonine phosphatase (PP1) and inhibitor of PP1 (I-1) (69). However, this mechanism is unlikely to completely explain the suppression of protein synthesis in the brain during torpor because the change in phosphorylation of eIF2α is only 11% and is not apparent in all tissues (93, 126). There is also increased phosphorylation of the elongation factor eEF-2 in hibernators (61). Increased phosphorylation of eEF-2 is typically associated with inhibiting the elongation phase of protein synthesis and appears to be orchestrated by increased eEF-2 kinase activity and decreased amounts of protein phosphatase 2A activity in hibernators. The resulting increase in eEF-2

phosphorylation could be responsible for the increased ribosomal transit times, i.e., lowered rates of translational elongation, that were observed in extracts prepared from torpid animals (61).

3. Mitosis and cell proliferation

DNA synthesis and cell division are energetically demanding processes. Not surprisingly, cell proliferation is severely depressed during torpor, which is consistent with the profound reduction in transcription and translation while hibernators are in the torpid state (93, 149, 242, 244). The most extensive studies on rates of cell division during hibernation have been carried out with the highly proliferative tissues of the gastrointestinal tract (1, 158). DNA synthesis in small intestinal epithelial cells continues during torpor at ~4% of rates in active animals (158); however, mitotic activity ceases during deep torpor (158). Intestinal epithelial cells (enterocytes) progress from the G_1 to S phases of the cell cycle during torpor but are apparently blocked in G_2 or late S (1, 158). It is not clear if low temperature is wholly responsible for the block in the cell cycle during torpor or if the G_2 arrest is a regulated response that minimizes errors in cell replication that might occur at low or variable temperatures (157). Mitotic activity and cell proliferation subsequently resume during periodic arousals (1, 53, 158).

Rates of enterocyte proliferation during interbout arousals for most (but not all, Ref. 230) hibernators are similar to or exceed those recorded in the summer-active state (1, 80). This is somewhat surprising, because the absence of food intake during the hibernation season would be expected to reduce enterocyte proliferation regardless of T_b due to the strong link between food intake and mucosal growth (103). On the other hand, high rates of cell proliferation during interbout arousals are consistent with the observation that protein synthesis on a whole body level is higher shortly after ground squirrels have aroused from torpor compared with squirrels that had been active for 1–2 days (264). The normal migration of enterocytes from the proliferative zone in the crypts to the villus tips is also greatly suppressed during torpor and resumes shortly after T_b begins to rise during a periodic arousal (53). However, the duration of typical interbout arousals (<24 h) prevents most cells from reaching the villus tips, and cells are arrested in the position they held when torpor resumes. Full turnover of villi can take as long as 3 wk in a hibernating squirrel compared with the typical turnover time of 3–5 days for nonhibernators (including summer squirrels). Although "physiologically young," enterocytes of hibernating mammals are chronologically much older than the typical enterocyte of a nonhibernator and represent an intriguing natural model for cellular aging.

4. Ultrastructural changes

Although there is anatomical evidence for structural changes in certain cells and subcellular compartments over the course of the annual cycle of hibernators, the functional significance of such changes for cellular physiology and gene expression has largely remained elusive. For example, cell nuclei of liver, pancreas, BAT, and adrenal cortex of dormice contain nuclear bodies that are present during torpor and disappear upon arousal. The structures, which bear similarity to heterogeneous ectopic ribonucleoprotein-derived structures (170–172), have been suggested to serve as storage/assembly sites of molecules needed for rapid resumption of transcriptional and posttranscriptional activities upon arousal from torpor (170, 171). Azzam et al. (10) recently described cytoplasmic "slits" within the endoplasmic reticulum of neurons and certain other cell types in hibernating ground squirrels. These structures were suggested to represent protein-free domains in which ribosomes and the cytoplasmic matrix had been displaced, possibly in response to intrinsic phase behavior of membrane lipids that display gelation and subsequent exclusion of proteins.

5. Membrane composition and function

Homeoviscous adaptation refers to a remodeling of membrane composition in response to changes in T_a that have the potential to alter membrane fluidity, such that membrane function is preserved. This response, which is common in certain poikilothermic species such as fish, would seem to be a useful component of the phenotype of hibernating mammals. Yet, despite many studies that have compared membrane characteristics in hibernators with their active counterparts, there is little evidence for a consistent pattern of changes in composition or fluidity of plasma or intracellular membranes that might facilitate the persistence of membrane function at the low temperatures of torpor (reviewed in Ref. 2). For example, an increase in membrane fluidity at low T_b might be expected to involve an increase in the proportion of membrane phospholipids that is unsaturated. Whereas this seems to be the case for some organs (e.g., brain), there is either no change or in some cases a decrease in the unsaturation index during hibernation for other tissues. The changes in membrane lipid composition that have been observed may be the consequence of altered phospholipid metabolism at low temperature, rather than a result of adaptive alterations that increase membrane fluidity during torpor (2). Moreover, it is questionable whether the small changes in the lipid-phase transition temperature in certain membranes (such as the inner mitochondrial membrane), which correlate with changes in phospholipid polar head groups and fatty acid composition, are physiologically relevant and can fully account for the changes in the Arrhenius plot breaks that have

been observed during hibernation compared with active animals (200). Thus, although membrane compositional changes in hibernating mammals may have other functional roles, the classic homeoviscous adaptation exhibited by poikilothermic organisms is apparently not a component of the hibernating phenotype (2). It is unlikely that membrane fluidity is compromised during torpor despite the lack of evidence for generalized changes in membrane composition. However, there are no data to directly confirm or refute this idea.

Nonetheless, there is some evidence for hibernation-specific alterations in membrane-associated proteins. Moesin, a member of the ezrin-radixin-moesin (ERM) family of membrane cross-linkers, is expressed in the brush-border membrane of intestinal epithelial cells in torpid ground squirrels but not in summer-active squirrels nor in euthermic hibernators during periodic arousals (104). Moesin and the closely related protein ezrin provide structural and functional connections between the plasma membrane and the underlying actin cytoskeleton. In adult mammals, including 13-lined ground squirrels of all activity states, ezrin is found constitutively in the brush-border membrane of enterocytes. However, in torpid squirrels, moesin colocalizes with ezrin (104). Although moesin has not been previously detected in small intestinal epithelial cells of adult mammals, in the rat small intestine moesin mRNA levels increase during embryonic development and then drop off after birth. In contrast, ezrin mRNA levels continue to increase after birth until the villus epithelium has become morphologically differentiated (13). This suggests the intriguing possibility that expression of moesin during torpor reflects a transient return to the fetal condition, consistent with the extended period of inactivity of the digestive tract during this time. Because ERM proteins are involved in the formation of membrane protrusions such as microvilli, the induction of moesin during hibernation may play a role in the maintenance of intestinal microvilli during hibernation (56).

Disruption of plasma membrane ion gradients occurs in nonhibernator cells exposed to low T_b due to increased passive leaks of Na^+ and K^+ that are not matched by increased activity of ion pumps. This can lead to influx of Ca^{2+} into cells and inappropriate activation of Ca^{2+}-mediated signaling pathways and enzyme systems that damage membranes and intracellular proteins. How do hibernators guard against this potentially lethal event as they descend into torpor? Red blood cells from hibernating and nonhibernating species differ in their ability to maintain Na^+ and K^+ gradients at low temperatures (254). This appears to be due to reduced passive efflux of K^+ and influx of Na^+ at 5°C, as well as maintenance of higher relative rates of Na^+-K^+-ATPase pump activity compared with nonhibernators such as rats or humans (254). The mechanism for the better maintenance of pump activity at 5°C in hibernator cells is not known, but it does not appear to be due to pump affinity for ions or to pump number (i.e., density of oubain binding sites/cell). These are species-specific characteristics of red blood cells stored in the cold and do not necessarily represent a difference between active and torpid states within hibernator species. In fact, compared with the active state, Na^+-K^+-ATPase activity is depressed during torpor in skeletal muscle, kidney, and liver due to reversible phosphorylation of the enzyme (168), and there is no effect of torpor on Na^+-K^+-ATPase activity in heart (168) or intestinal epithelial cells (53).

The maintenance of ion gradients across cell membranes, and consequently cellular and organ function at low temperature, may be more effective than in nonhibernating species because of the superior regulation of intracellular Ca^{2+} concentrations displayed by the hibernators (111, 250). For example, upon chemical depolarization, brain synaptosomes isolated from torpid ground squirrels exhibit reduced accumulation of $^{45}Ca^{2+}$ compared with active squirrels that were cold-adapted, an effect that appeared to involve a reduction in the activity of Q-type calcium channels (101). Species differences in the response of cardiac sarcoplasmic reticulum (SR) to low temperature may account for the rise in cytosolic free Ca^{2+} in cold-sensitive species and may be responsible, at least in part, for the inability of cold-sensitive hearts to function at low temperature (164). A novel isoform of the Ca^{2+} binding protein calsequestrin is expressed in the cardiac SR of two hibernating species. Together with an altered ryanodine receptor/Ca^{2+} channel found in the heart of hibernators, these protein modifications may play a role in the ability of cardiac muscle of hibernators to regulate Ca^{2+} release from and storage in the SR at low T_b (178).

6. *Mitochondrial respiration*

Oxygen consumption in mitochondria is ubiqutious among all cells and accounts for a large proportion of metabolism. It has been known for some time that state 3 (fully coupled) respiration is depressed in mitochondria isolated from torpid hibernators, and suspected that this depression is reversed upon each interbout arousal (see Ref. 174 and references therein). Inhibition of state 3 respiration was confirmed for liver mitochondria from golden-mantled ground squirrels, then tested in animals entering into or arousing from torpor (174). The inhibition did not occur until the animals were already torpid, suggesting that this is not a crucial component of lowering metabolic rate during entrance into hibernation. Furthermore, the inhibition was not removed as the animals began to arouse from torpor, which would be required if reversal of the inhibition drives the arousal process. In fact, the findings were most consistent with the appearance of a state 3 respiration inhibitor during torpor. It will

be interesting to determine the nature of this inhibition and its significance for arousal. Finally, this study did not find any evidence for differences in state 4 respiration (proton leak) among any of the hibernation stages (174), although significant reductions in both state 3 and state 4 respiration were reported recently for liver mitochondria isolated from hibernating arctic ground squirrels (12). The reduction in state 4 respiration was not due to a change in the proton permeability of the mitochondrial membranes, but rather to a reduction in the activity of the reactions that generate the proton gradient. Neither state 3 nor state 4 respiration was altered in mitochondria from skeletal muscle examined in the same study (12).

7. Digestive function

Solute transport across the small intestinal epithelium is greatly suppressed at the low temperatures of torpor, which is consistent with thermal effects of cooling on transport kinetics. However, nutrient and electrolyte transport rates increase rapidly when tissues are rewarmed in vitro and at 37°C are similar to those in summer animals (46–48). Thus the molecular and biochemical machinery to carry out active transport is apparently well preserved in hibernating mammals despite the drastic reduction in gut mass that occurs during the long-term fast (46, 47, 94, 134). In 13-lined ground squirrels, hibernation has little or no effect on the brush-border proteins sucrase-isomaltase, aminooligopeptidase, intestinal alkaline phosphatase, and the sodium-glucose transporter (SGLT1) with regard to mRNA or protein abundance, or enzyme specific activity (53). Similarly, hibernation has minimal or no effects on membrane-bound hydrolytic enzymes in European hamsters (53). The mRNA abundance and activity of Na^+-K^+-ATPase, which drives Na^+-coupled solute uptake into enterocytes, are also preserved in ground squirrels during hibernation (53). In fact, sugar and amino acid absorption are actually enhanced in the hibernating gut when normalized to mucosal mass or protein (46). These observations may reflect an evolutionary strategy that reduces biosynthetic costs during the winter months through a reduction in total intestinal tissue mass while maintaining the ability to resume digestive functions upon rewarming. In contrast to the minimal effects of hibernation on intestinal brush-border enzymes, the activity and protein expression of the pancreatic enzyme α-amylase are reduced by ~50% in hibernating 13-lined ground squirrels compared with values in summer squirrels (11). This is consistent with the strong influences of fasting and dietary carbohydrates on regulating pancreatic amylase levels in nonhibernating species.

Microbial fermentation in the hindgut of alpine marmots appears to continue at a basal level during hibernation, based on concentrations of short-chain fatty acids in hindgut segments measured shortly after spring emergence and throughout the active season (134). In 13-lined ground squirrels, there is some reduction in numbers of viable bacteria in the cecum during hibernation (hibernators were without food for 6 or 42 days), but overall, the cecal microflora remained remarkably stable (16). There are significant changes in the pattern of distribution of myenteric neurons in the gut of active and hibernating hamsters. Numbers of myenteric neurons immunoreactive for serotonin are reduced during hibernation, whereas those containing tyrosine hydroxylase, substance P, calcitonin gene-related peptide, and vasoactive intestinal polypeptide are increased (238). The functional significance of these changes has yet to be identified. The chipmunk, a food-storing hibernator, appears to maintain some degree of digestive function during bouts of torpor, presumably to allow efficient assimilation of food that is ingested during its periodic euthermic intervals (135).

8. Renal function

During torpor, renal blood flow, glomerular filtration rate, and urine formation cease in small hibernators (e.g., ground squirrels, dormice) or are greatly reduced in larger hibernators (marmots) (184, 263). These processes resume during periodic arousals, concomitant with the rise in T_b and cardiac output. Thus there are only moderate effects of hibernation on plasma osmolality and electrolyte concentration, and the renin-angiotensin system appears to be functional during periodic arousals (3, 267). Although ultrastructural changes in renal morphology during the annual cycle have been reported (3, 267), there is little information regarding changes in renal function at the molecular level.

9. Immune function

The immune system is unable to mount an effective response against an injection of bacterial lipopolysaccharide during torpor, but full restoration of the response occurs during interbout arousal (41, 204). A similar pattern is seen with classical complement activity, which is also suppressed during torpor and fully restored during interbout arousal (173). Immune status appears to influence torpor duration in Turkish hamsters. Hamsters that were previously challenged with antigen (hen eggwhite lysozyme) spent more time in torpor than control animals that were exposed to the same T_a and photoperiod but were not challenged (41). In addition, antigen-exposed hamsters that had hibernated had a suppressed humoral response to a secondary challenge after they were aroused and returned to warm conditions compared with challenged hamsters that were never allowed to hibernate. Thus hibernation appears to influence the immune system, and vice versa.

B. Processes That Continue Function at Low Temperature

1. CNS

Significant aspects of CNS function must continue during torpor, despite dramatically reduced electrical and metabolic activity (119, 145). This is exemplified by regulation of T_b around a progressively lowered setpoint during entrance into torpor and its continuous regulation throughout the torpor bout; the maintenance of a respiratory and cardiac rhythm during torpor; the sensitivity of torpid animals to external stimuli such as touch, light, and temperature; and the reversal of metabolic depression when periodic arousals are initiated, which presumably involves neurohumoral cues.

A dramatic demonstration of continued CNS function during torpor is seen in laboratory measurements of core T_b and metabolic rate during hibernation in arctic ground squirrels across a wide range of T_a values (−16 to 20°C) (34). When T_a was greater than or equal to 0°C, the average T_b was within 1°C above T_a, whereas at T_a equal to −4, −8, or −16°C, the average T_b was −0.42°C. Metabolic rate was at its minimum when T_a was 4°C. As T_a decreased below 0°C, metabolic rate increased. The increase was positively correlated with the difference between T_a and T_b, indicating the animals were increasing metabolic heat production to maintain a constant T_b as T_a declined. Alpine marmots also maintain a minimal metabolic rate between temperatures of 5 and 15°C and respond to higher and lower T_a values by increasing metabolism (198), but smaller animals are not able to enjoy this relative large temperature span of minimal metabolic rate. Rather, metabolism increases with increasing T_a from 5 to 10°C in torpid golden-mantled ground squirrels (266), and above about ~8°C in the edible dormouse (256). In all cases, however, the hibernating mammal remains fully capable of sensing changes in T_a and controlling T_b by metabolic heat production.

Seasonal hibernators display strong circannual rhythms in feeding behavior, body fat deposition, and body weight as well as metabolism that are closely associated with the annual reproductive cycle (201). The rhythms are endogenous; thus they are entrained, but not triggered by environmental cues, as evidenced by their persistence in constant conditions (75). These rhythms run independently: circannual reproductive cycles persist in the absence of hibernation in marmots (67), and early expression of reproduction does not disrupt the timing of the following year's body mass peak or reproductive maturation in ground squirrels (15). However, hormonal states influence the hibernation cycle (202), and vice versa (15). Entrainable circannual rhythms are also apparent in the amounts of serum thyroxine, prolactin (68), and leptin in woodchucks (66) that are held at constant T_a of 20–23°C throughout the year with food and water available ad libitum. The addition of exogenous leptin disrupts the normal rhythm of feeding behavior and body weight gain in arctic ground squirrels (30, 197). Levels of some brain neuropeptides (e.g., thyrotropin-releasing factor, β-endorphin) are lower during hibernation compared with the summer-active state, whereas others are increased (e.g., vasopression, enkephalin) (reviewed in Ref. 190). However, the circannual control of these molecules has not been specifically examined.

Compared with major advances in understanding the molecular basis of circadian rhythms in mammals (199), circannual rhythms remain obscure at the cellular and molecular levels. Circadian organization is abolished under natural conditions of hibernation (constant darkness in the burrow) (137, 159), although hibernators remain sensitive to expression of circadian rhythms entrained by light and possibly T_a (105, 153). In ground squirrels, reappearance of typical circadian rhythms occurs gradually after final emergence from torpor in the spring, providing further evidence that the circadian system is suppressed during the hibernation season (137). Regain of summer function is correlated with an increase in arginine vasopressin immunoreactive neurons in the suprachiasmatic nucleus (SCN) (138). Studies of hibernation in three hamster *tau* variants provide a particularly strong demonstration of the dissociation of circadian and circannual rhythms in hibernation. The three *tau* genotypes exhibit free-running circadian rhythms of 20, 22, and 24 h, respectively. If onset of torpor or arousal were under the control of the circadian system, there should be differences in the timing of torpor among the three *tau* strains; however, no differences were detected (194). However, experiments to ablate the SCN of the hypothalamus, a crucial structure for circadian rhythms, do support a role for the circadian system in the control of timing and duration of torpor bouts. SCN-lesioned golden-mantled ground squirrels exhibit greater variability in the duration and timing of their torpor cycles than do normal controls (209, 210).

2. Cardiovascular system

One of the hallmarks of hibernation is the continued and regulated contractile function of cardiac muscle at the low T_b values of deep torpor (179). In contrast, hearts of nonhibernators are much more sensitive to cold. They display arrhythmic patterns beginning at 32°C, begin atrial fibrillation at 30°C, and display increased ventricular excitability with asystole and cardiac arrest at 15°C (for review, see Ref. 141). The cellular and molecular bases for the continued function of cardiac tissue at low T_b is unknown. However, changes in metabolic fuel use in heart during hibernation may play a role in continued function at low T_b values. Belke et al. (20) measured the effects of temperature on glucose and fatty acid (palmi-

tate) oxidation in hearts isolated from Richardson's ground squirrels in their nonhibernating or hibernating stages. Hearts taken from both groups of squirrels were perfused at 37 and 5°C. Glucose oxidation rates were significantly lower in the hibernating heart compared with the nonhibernating heart at both temperatures. When glucose oxidation and palmitate oxidation were viewed in terms of their percentage contribution to tricarboxylic acid cycle activity, the hibernator was more reliant on palmitate oxidation as its source of acetyl CoA at both temperatures. When the hibernator and nonhibernator were compared, at both temperatures the contribution of palmitate oxidation to acetyl CoA production was higher in the hibernator than in the nonhibernator, although this difference was slight at 37°C. Thus, while the percentage contribution that fatty acid oxidation makes to acetyl CoA production is slightly decreased in the hibernator at the lower temperature, the hibernator is still >80% reliant on fatty acid as its source of energy at 5°C.

3. Ventilation

Ventilation rate is markedly reduced during torpor due to a reduction in breathing frequency that occurs in parallel with the fall in metabolism; both events precede the fall in T_b as entrance into torpor progresses. Extended periods of apnea can occur during deep torpor that last from minutes to hours. Upon arousal, episodic breathing disappears and breathing frequency rapidly resumes its normal, active pattern (176). The cellular basis for the change in the ventilation pattern during torpor-arousal cycles is unknown but may involve changes in the intrinsic properties of the respiratory rhythm generator in the brain stem (177).

4. Adipose tissue

Both WAT and BAT must remain responsive and retain function at the low T_b values of torpor. WAT is the dominant metabolic fuel throughout the hibernation season, and the quantity and quality of these fat stores are apparently assessed because there are consequences on the timing and patterns of torpor (87, 90, 99, 113, 124, 205). Alpine marmots maintain a constant fatty acid composition in WAT across the annual cycle, despite differences between the WAT composition and the fatty acid composition of their diet and the preferential release of certain fatty acids from isolated adipocytes stimulated in vitro (64). Seasonal differences are apparent in the responses of gonadal versus subcutaneous adipocytes to various agents that stimulate lipolysis (65). There are also seasonal differences in the activities of enzymes involved in lipogenesis and triacylglycerol synthesis in some tissues (183, 249). Lipolysis in WAT at low T_b is facilitated by expression of PTL in white adipocytes because PTL retains a greater activity at low temperatures compared with hormone-sensitive lipase (17). It will be important in the future to consider other aspects of WAT function in hibernators; there has been a rapid increase in information indicating that the roles of WAT extend beyond its long-appreciated functions in energy storage to larger roles as a major secretory and endocrine organ in energy homeostasis (21, 240). Leptin was the first hormone found to be secreted from adipocytes, and it appears to act as a signal to reduce food intake when adequate fat stores are present. Seasonal hibernators often double their body weight in a short period just before hibernation, which requires adjustments in the normal mechanisms of body weight regulation so that large stores of fat can be acquired. Indeed, there appears to be dissociation between leptin secretion from adipocytes and adiposity as little brown bats fatten before the onset of hibernation (156). Another potent physiological effector secreted from adipocytes, adiponectin, has not yet been studied in hibernators, but it is intriguing that it is homologous to proteins first identified as declining during hibernation in Siberian chipmunks, HP-20, HP-25, and HP-27 (21).

In addition to WAT, placental hibernators also utilize BAT for nonshivering thermogenesis at low T_b mediated by UCP-1. Nonshivering thermogenesis in BAT is also likely responsible for the heat generation that maintains T_b when decreases in T_a have the potential to reduce T_b below setpoint temperature during steady-state torpor in placental hibernators (34). However, because BAT is generally not found in marsupial and monotreme hibernators (115, 131), it must not be essential for maintenance of T_b during hibernation or for arousal from hibernation. Two UCP homologs were reported to be upregulated at the mRNA level during hibernation in BAT and other tissues (29). Whether these contribute to enhanced thermogenesis in BAT (29) or the other tissues is unknown. Although UCP2 and UCP3 appear to be involved in energy balance, they do not appear to be directly involved in adaptive thermogenesis (5, 102). Other potential roles for these UCPs have been proposed (5), including the regulation of reactive oxygen species (ROS) (6).

VI. CELLULAR STRESS AND STRESS TOLERANCE

Many aspects of hibernation are highly stressful and even lethal for mammals that do not hibernate. This has long been recognized at the organ and whole body levels for such processes as cardiac function, which is severely impaired in nonhibernators at the low T_b values typical of torpor (38, 141). More recently, evidence has been accumulating to suggest that hibernation also increases the risk of one or more forms of cellular stress, and the adaptive responses hibernators possess to combat cellular stress are beginning to emerge. Extended periods of

low temperatures can be stressful to cells due to effects on such processes as protein stability, membrane function, ATP synthesis, activity of key regulatory enzymes, and cytoskeletal integrity (142, 217). In fact, incubation of cells from nonhibernating species at the low temperatures that characterize the torpid state can induce the expression of stress (heat shock) proteins (71, 217). In addition to thermal stress, hibernators are vulnerable to metabolic stress that can result from low tissue perfusion during torpor as well as lack of dietary sources of defense molecules, such as antioxidants and their precursors. Thus hibernators are increasingly being recognized as a valuable natural model for the evolution of endogenous defense mechanisms in response to naturally induced stress (49, 58, 78, 91, 92). In this section, we highlight the evidence for cellular stress pathways and stress tolerance in mammalian hibernators.

It has been proposed that hibernators experience ischemic events during the hibernation season based on the highly variable rates of tissue perfusion during torpor-arousal cycles as well as the prolonged periods of apnea that occur during torpor in some hibernating species (36, 92, 176). However, because metabolism and T_b tend to fall during torpor in parallel with reduced tissue perfusion, it is likely that the greatly reduced delivery of nutrients and oxygen to cells during torpor is effectively matched by the reduced energetic demand of the metabolically suppressed tissues. Furthermore, rates of deleterious processes normally associated with ischemic stress, such as production of free radicals and oxidative attack on cellular macromolecules, are probably also lower in torpid compared with active animals because of the low T_b values. That said, hibernators may still be at risk for ischemic damage during entrance into torpor because heart rate and cardiac output fall before a significant reduction in T_b as torpor progresses (179); this may result in reduced tissue perfusion in areas that are still undergoing biochemical reactions at euthermic rates. Similarly, the rapid increase in T_b during periodic arousals to euthermia may increase the risk of regional ischemia-reperfusion injury in areas where blood flow is not fully restored before elevations in T_b. The large burst in mitochondrial respiration during arousal may also exceed the limits of antioxidant defense systems typical of homeotherms that do not normally experience such rapid changes in mitochondrial activity. Changes in tissue perfusion that take place during torpor-arousal cycles may be particularly critical for the brain; cerebral blood flow is reduced during torpor to about one-tenth the rate of active animals (92).

Is there direct evidence for oxidative stress associated with hibernation? Studies with hibernating arctic ground squirrels indicated that plasma urate levels peak at the time of maximal oxygen consumption during arousal and are lowest in torpid squirrels (237). Because urate is produced when xanthine dehydrogenase is converted to xanthine oxidase by oxidative stress, this pattern likely reflects increased production of ROS during arousal and reduced ROS production during torpor. Urate content in peripheral tissues is also much lower during torpor compared with nonhibernating squirrels or those recently aroused from torpor (237).

Concentrations of conjugated dienes in the intestinal mucosa are significantly greater in 13-lined ground squirrels during the hibernation season compared with levels in summer-active squirrels (50). Among hibernation states these lipid peroxide metabolites are greater in early torpor (within ~24 h of entering a torpor bout) compared with squirrels arousing from torpor. Because the intestine is one of the most poorly perfused organs during torpor (5% of normal rates) and one of the last to receive normal flow upon arousal (36), the increased lipid peroxidation in the gut during hibernation may reflect increased production of ROS and subsequent damage to cellular lipids induced by low tissue perfusion and/or reperfusion during the torpor-arousal cycle. Conjugated diene concentrations in WAT of white-tailed prairie dogs are twofold greater than in the related black-tailed prairie dog, which may reflect the much greater depth and duration of torpor bouts expressed by the former species (113). Increased lipid peroxide levels in plasma and erythrocyte membranes have also been reported in hibernating bears whose T_b values were only minimally reduced (~2°C) from euthermic summer levels (60). This suggests that oxidative stress in hibernators may not be due to thermal effects per se but is more likely related to metabolic depression or some other aspect of the hibernating phenotype.

Studies in arctic and 13-lined ground squirrels suggest that plasma ascorbate may function as an antioxidant during the hibernation season (77, 237). Plasma ascorbate increased three- to fourfold in both species during torpor and returned to euthermic levels upon arousal, an effect that appeared to be due to changes in oxygen consumption rather than T_b (77). In contrast, tissue ascorbate concentrations increased significantly during arousal in liver and spleen, which may reflect a redistribution of plasma ascorbate pools to counter increased ROS production generated by the rapid increase in mitochondrial activity during this time (237). The activities of several antioxidant enzymes are also increased in certain tissues of European ground squirrels during hibernation. BAT, which undergoes dramatic increases in mitochondrial activity and blood flow during arousal, displayed higher activities of superoxide dismutase, ascorbate, and glutathione peroxidase during hibernation. Glutathione peroxidase was also increased in liver, and ascorbate levels were higher in plasma during hibernation (42–44).

Hibernation is also associated with changes in glutathione redox balance in the gastrointestinal tract. Gluta-

thione is the major thiol-disulfide redox buffer within cells and, in conjunction with reduced glutathione redox cycle enzymes, plays a key role in the detoxification of endogenous and exogenous ROS including lipid peroxides (8). Cellular oxidative stress is often manifested as a shift in the ratio of glutathione from its reduced (GSH) to oxidized (GSSG) forms, with lower ratios reflecting a more oxidized state. The GSH-to-GSSG ratio in intestinal mucosa of hibernating 13-lined ground squirrels is 5-fold lower than in summer animals, an effect due primarily to elevated GSSG concentrations during hibernation. The activity of GSSG reductase, which catalyzes the regeneration of GSH from GSSG, is reduced by ~50% in intestinal mucosa during hibernation and thus may play a role in the inability of the gut to maintain normal GSH-to-GSSG ratios (54). One factor that may compromise maintenance of redox balance during hibernation is nutritional stress. The intestine relies on dietary or biliary derived GSH and GSH precursors for maintenance of mucosal GSH (7), and both sources would be compromised by extended fasting. Furthermore, enterocytes require exogenous glucose for NADPH production to maintain function of the GSH redox cycle, and this may be limited by decreased availability of luminal glucose in fasted hibernators (9). Interestingly, the concentration of total GSH equivalents ([GSH] + 2×[GSSG]) within the hibernation season in intestinal mucosa is lowest in squirrels arousing from hibernation and highest in euthermic squirrels during an interbout arousal (54). This raises the possibility that periodic arousals to euthermia may allow at least partial restoration of the total GSH pool and serve to minimize severe changes in GSH redox balance, possibly via luminal delivery of GSH and GSH precursors in bile. The change in intestinal GSH redox balance during hibernation does not appear to be due to changes in T_b but rather to other consequences of metabolic depression. For example, GSSG concentration and the GSH-to-GSSG ratio in euthermic hibernators during an interbout arousal are very different than in summer squirrels that are at the same T_b (~36°C) (54).

In contrast to these findings, other studies reported little or no effect of hibernation on tissue GSH levels (77, 237), although the lack of measurements of GSSG concentrations makes it difficult to assess hibernation-induced changes in the total GSH pool or in GSH-to-GSSG ratios. However, the observations for the gut in mammalian hibernators are very similar to those reported for the intestine of desert toads during estivation, a state that is also characterized by fasting and metabolic depression. Conjugated diene levels were elevated 39% in the gut of estivating toads compared with awake animals, and the GSH-to-GSSG ratio was lower during estivation due to higher GSSG concentration and no change in GSH levels (108). Thus oxidative stress and redox imbalance may be a general feature of animals that undergo metabolic depression.

Ubiquitin is a highly conserved 14-kDa polypeptide that when conjugated to cellular proteins tags them for degradation by the 26S proteasome. An increase in the abundance of proteins conjugated to ubiquitin in tissues has been used as an indicator of the extent of protein damage induced by stress (129). In gut and liver of two species of ground squirrels, protein-ubiquitin conjugate concentrations increased during entrance into torpor and were elevated two- to threefold by late torpor compared with levels in nonhibernating squirrels in spring or summer (241). Ubiquitin-conjugate levels in arousing or interbout arousal squirrels were similar to those in summer squirrels (241). Thus increased rates of protein damage associated with entrance into a metabolically depressed state may lead to accumulation of ubiquitylated proteins as animals enter torpor. An alternative interpretation of these findings is that ubiquitin-mediated proteolysis is suppressed at the low T_b of torpor which results in the accumulation of ubiquitylated proteins. Interbout arousals during the hibernation season may subsequently allow for degradation of these conjugated proteins and the restoration of functional protein pools.

Cellular stress and redox imbalance can induce expression of stress proteins and other stress-activated signaling pathways. Is there evidence for induction of the stress response during hibernation? Induction of the most well-studied stress protein, the 70-kDa heat shock protein (HSP70), occurs in response to a wide variety of events including heat and cold stress, osmotic stress, pH change, as well as oxidative stress and changes in cellular redox balance (189, 252). Results of studies that measured expression of HSP70 in hibernators are variable, which may reflect tissue specificity and the point in the annual cycle when studies were conducted. HSP70 mRNA and protein were either not affected or reduced in hibernating squirrels during torpor and interbout arousal compared with euthermic animals in brain, heart, liver, kidney, skeletal muscle, and BAT (23, 57). In the intestine, HSP70 protein levels are similar in active and torpid squirrels (57), but differences exist within the hibernation season. Intestinal HSP70 levels are highest in squirrels entering torpor and early in a torpor bout and lowest in squirrels arousing from torpor and during an interbout arousal period (54). There were no differences reported in the expression of the constitutive HSP70 gene family member, HSC70, in brain, heart, and kidney of euthermic and torpid bats (226).

Expression of glucose-regulated protein 75 (GRP75), a member of the HSP70 family of stress proteins, is higher in liver, gut, and skeletal muscle of hibernating ground squirrels compared with summer-active animals (50, 57). GRP75 is expressed constitutively in mitochondria where it chaperones the translocation of nuclear-encoded pro-

teins into the inner mitochondrial matrix (22, 180). Another mitochondrial stress protein, HSP60, is not altered by hibernation (57). The induction of GRP75 during hibernation may be another indicator of oxidative stress during the hibernation season because in nonhibernators GRP75 has been shown to be induced by stressors such as glucose depletion and ischemia (175, 180). Thus hibernators may rely on increased levels of stress proteins such as GRP75 during torpor-arousal cycles to maintain mitochondrial integrity and minimize oxidative stress induced by rapid changes in mitochondrial activity and ROS production. Increased expression of GRP75 as well as the stress proteins GRP94 and GRP78 occurs in brains of bats 30 min into arousal from torpor when body temperature increases from 7 to 35°C and oxygen consumption increases approximately ninefold (162). Concomitant with these changes are increases in activity or expression of the stress-activated kinase JNK and the prosurvival proteins Akt and protein kinase C-γ. These effects may be components of a neuroprotective mechanism that helps maintain brain function and whole organism survival during repeated cycles of torpor and arousal.

Other evidence that mitochondria may be particularly sensitive to stress and respond adaptively during the hibernation season is indicated by results of a differential screen for upregulated genes in a ground squirrel kidney cDNA library in which increased expression of mitochondrially encoded cytochrome-*c* oxidase subunit 1 (Cox1) was detected (127). Subsequent Northern analysis supported differential expression of mRNAs for Cox1 as well as ATP synthase 6/8 in kidney, heart, and BAT. Because enzymes involved in mitochondrial electron transport are known to be sensitive to ischemia-reperfusion injury in nonhibernating species, increased expression during hibernation of certain mitochondrial proteins may be a mechanism to minimize damage to the electron transport chain caused by extended low body temperatures or ischemic events that may occur during torpor-arousal cycles (127).

Implantation of microdialysis probes into striatum of arctic ground squirrels during hibernation and euthermy were used to determine whether hibernation afforded protection against traumatic stress to the brain (265). Immunocytochemical detection of the stress protein heme oxygenase-1 (HO-1) around probe sites was used as an indicator of oxidative stress. HO-1 immunoreactivity was present around probe sites in the active squirrels but was absent in hibernators, which was interpreted as evidence that hibernation afforded protection against injury-induced oxidative stress.

Another stress-activated protein whose expression is altered by hibernation is the transcription factor nuclear factor-κB (NF-κB). Nuclear translocation of NF-κB is greater in intestinal mucosa from hibernating ground squirrels compared with summer animals (50). Within the hibernation season activation of NF-κB in the gut is evident as animals enter torpor, remains high throughout a torpor bout, and is lowest in hibernators arousing from torpor (50). This pattern is not common to all tissues because BAT displays little evidence of basal NF-κB activation in either active or hibernating squirrels (50).

In cells of nonhibernating species NF-κB activation is induced or enhanced by pro-oxidative shifts in the GSH redox state, particularly when GSSG concentration is elevated (79). Thus increased activation of NF-κB in the gut during hibernation is consistent with higher levels of GSSG during this time (54). Although in nonhibernators NF-κB activation is associated with a variety of gastrointestinal diseases such as ischemia-reperfusion injury and inflammatory bowel disease, whether its target genes contribute to disease pathology or exert protective effects varies with the particular condition and the timing of NF-κB expression. Because hibernation does not impart overt damage to the gut, it is likely that NF-κB activation plays a protective role during hibernation. Many of the gene products induced by NF-κB are involved in antioxidant defense, recruitment of immune cells to specific sites via production of cytokines and chemokines, and regulation of cell proliferation and apoptosis. All of these pathways may be important for the hibernating phenotype, at least for the gut. Preliminary evidence indicates that enterocyte apoptosis increases during hibernation (49), which suggests that activation of NF-κB target genes may play antiapoptotic roles and thereby promote enterocyte survival during this time. There is also a marked increase in lamina propria and intraepithelial lymphocytes in the mucosa during hibernation but little evidence of infiltration of polymorphonuclear leukocytes or other immune elements that are associated with inflammation (49). The maintenance of the mucosal immune system during hibernation contrasts with the systemic leukopenia that is characteristic of the torpid state (92, 237). The cause of hibernation-induced leukopenia is not known but may be a consequence of increased levels of plasma cytokines or chemokines that influence leukocyte trafficking, because incubation of rat endothelial cells with plasma from hibernating ground squirrels increased expression of the cell surface adhesion molecule intercellular adhesion molecule-1 (ICAM-1) to a greater extent than did plasma from active squirrels (261). Incubation with plasma from hibernating and to a lesser extent, nonhibernating squirrels also increased adherence of monocytes to rat endothelial cells (261). Because circulating leukocytes are closely associated with pathogenic mechanisms that mediate ischemia-reperfusion injury, it has been suggested that the leukocytopenic state of torpid animals may reduce the risk of inflammatory infiltration of vulnerable areas like the CNS during the arousal process (261).

Mammalian hibernators display several features that indicate they adaptively respond to endogenous stress as part of the annual cycle of extreme changes in T_b, metabolism and other physiological processes. Although it is still not clear whether tissues of hibernators experience true ischemia during cycles of torpor and arousal, there are good indicators that at least some tissues are vulnerable to stress, particularly oxidative stress, during hibernation. The lack of overt damage to tissues during hibernation suggests that redox shifts and oxidative stress are not deleterious events but instead may be part of an adaptive defensive strategy that allows hibernators to withstand physiological extremes during the winter months. Indeed, the fact that the shift in intestinal GSH redox balance to a more oxidized state during hibernation is maintained at a relatively constant level throughout the hibernation season (54) suggests that maintenance of altered redox homeostasis may be part of the suite of adaptations that comprise mammalian hibernation. It is intriguing to consider that a moderate degree of oxidative stress produced at one point during torpor-arousal cycles (e.g., entrance) may activate defensive pathways that minimize stress that will be incurred during a subsequent phase of the cycle (e.g., arousal) (Fig. 4). In this scenario, redox changes during torpor-arousal cycles may act to "precondition" tissues for activation of defensive pathways that increase tolerance to physiological stress, as has been suggested from studies in anoxia-tolerant lower vertebrates (122). The observation that ground squirrels fed diets high in α-tocopherol, an important ROS scavenger, are less likely to enter torpor and less likely to survive torpor (89) also supports the idea that a minimum level of oxidative stress may actually be required for normal hibernation patterns. More intervention-driven studies are needed to test these ideas directly.

VII. COMPARISON OF MAMMALIAN HIBERNATION WITH OTHER HYPOMETABOLIC STATES

Lessons from the differential expression of genes controlling carbon utilization in mammalian hibernators have revealed similarities with hypometabolic states in organisms with well-defined genetics. Similarities among the expression of PDK4 during hibernation, the activation of PDK4 in diabetic (low insulin) rodents (258, 260), and the reduction in glucose-based metabolism due to a defective insulin-like receptor in dormant nematodes (146), suggest a conserved mechanism for the molecular control of carbon utilization in mammalian hibernation and nematode diapause**.** Diapause in the nematode *Caenorhabditis elegans* is characterized by increased fat accumulation and arrested development resulting in the dauer larva stage. For periods of up to 6 mo dauer larvae do not feed, show no movement, and undergo a reduction in glucose-based catabolism (reviewed in Ref. 206). When environmental conditions become more favorable, as seen with spring emergence in hibernating mammals, dauer larvae will resume development and are nearly indistinguishable from individuals that have not arrested at the dauer stage. Genetic analysis has shown that a protein homologous to the mammalian insulin/insulin-like growth factor (IGF-I) receptor, called DAF-2, is a key player in the switch from a normal life cycle to the arrested dauer stage (146). Dauer larvae formation will occur constitutively when a

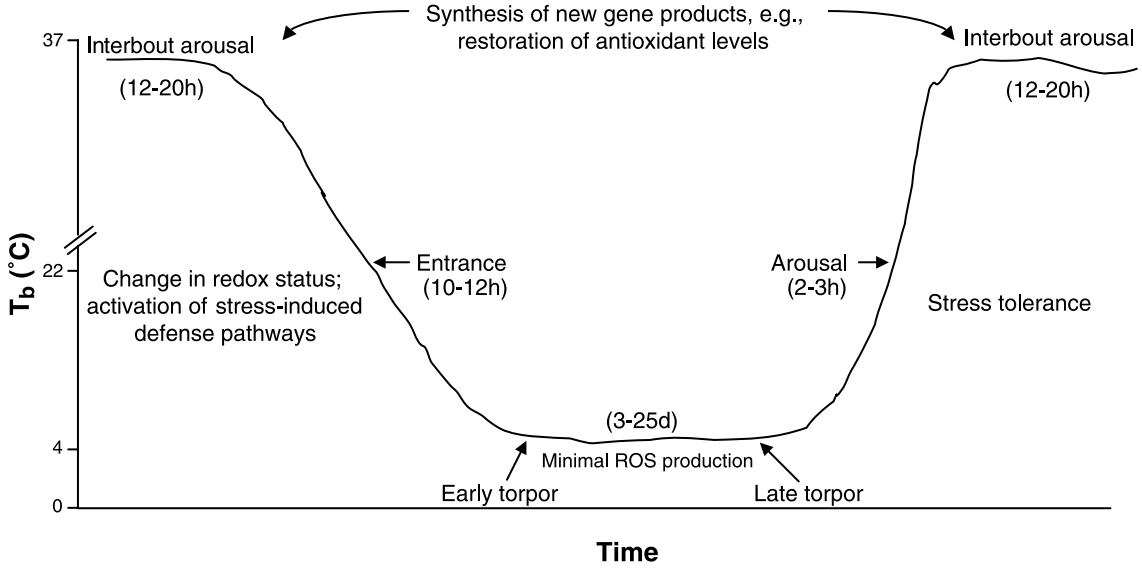

FIG. 4. Model of stress induction and tolerance during torpor-arousal cycles in hibernators, based on intestinal mucosa. Numbers in parentheses indicate approximate durations of hibernation states.

mutation in the *daf-2* gene produces a receptor that is defective in the ligand-binding domain. Hence, the inability of an insulin-like ligand to bind DAF-2 leads to a state of dormancy and metabolic rate depression analogous to hibernation in mammals.

Genetic conservation between insulin signaling in mammals and diapause signaling in *C. elegans* is also seen early in the dauer signal transduction pathway where mutant *age-1* alleles cause a dauer constitutive phenotype. The AGE-1 protein is closely related to a family of phosphatidylinositol 3-kinase p110 catalytic subunits thought to associate with the insulin receptor and provide the next step of the insulin-signaling pathway in mammals (182). Partial activation of the dauer pathway by various *daf-2* and *age-1* mutants in *C. elegans* (reviewed in Refs. 59 and 116) results in an extension of life span similar to that seen in hibernating mammals (166). A 75% decrease in circulating IGF-I in hibernating golden-mantled ground squirrels (213) suggests a reduction in IGF-I signaling similar to that seen in the long-lived *C. elegans* mutants. In addition, knock-out mice that are heterozygous null for the IGF-I receptor live 26% longer than their wild-type littermates (130). It is possible that an element of the increased longevity associated with hibernation results from elevated PDK4 activity, which reduces carbohydrate oxidation and thus mimics the life-extending effects of caloric restriction in mammals (251).

Recently, *daf-2* mutants have also been shown to confer resistance to hypoxia in *C. elegans* (215). As with the reversibility of low oxygen consumption during arousal from hibernation in mammals, these hypoxia-resistant mutants (Hyp) fully recover from up to 20 h of extremely low oxygen concentrations (<0.3% O_2 at 28°C). This level of O_2 is lethal to wild-type *C. elegans*, just as the relative oxygen consumption of a mammal in deep hibernation is lethal to a nonhibernating mammal. Genetic analysis shows that the *daf-2*-mediated signaling pathway for hypoxia resistance is distinct from that involved in metabolic stasis and longevity (215). Nonetheless, it is interesting that various phenotypes associated with hibernation in mammals are also seen with mutants of a single gene encoding an insulin-like receptor in worms.

The physiological and biochemical changes that occur in mammalian hibernators are similar in some respects to those observed in lower vertebrates and invertebrates that undergo estivation and other forms of dormancy. These include the seasonal deposition of fuel reserves, typically in the form of lipid; the switch from carbohydrate to lipid oxidation during periods of metabolic depression; depression of RNA and protein synthesis and degradation; use of reversible phosphorylation of enzymes and other proteins as means to rapidly control metabolic processes; and evidence of increased oxidative stress and antioxidant defenses in certain tissues (108, 109, 112, 121, 227).

VIII. IMPLICATIONS FOR BIOMEDICINE

During their active phase, hibernating mammals display a physiological profile that is essentially identical to nonhibernating mammals. Yet, each year species that hibernate alter their phenotype and maintain new physiological states that would be lethal for nonhibernators, including humans. Better understanding of the regulated induction and reversal of metabolic depression, the resistance to deleterious effects of extended hypothermia, the adjustment to extended fasting, the finely tuned regulation of body weight gain and loss, and other key characteristics of mammalian hibernation hold great promise for translation to the clinical arena. Here we highlight some of the potential applications of hibernation biology to biomedicine.

A. Ischemia/Reperfusion Injury

Mammalian hibernators represent a unique natural model for resistance to the deleterious effects of low tissue perfusion and reperfusion. During deep torpor, respiration is reduced from 100–200 to 4–6 breaths/min, heart rate is reduced from its normal 200–300 to 3–5 beats/min, and blood flow is preferentially shunted away from splanchnic organs and the periphery to provide nutrients and oxygen to critical organs that must function at very low temperatures, such as brain, lungs, heart, and adipose tissue. Upon arousal to euthermic T_b, metabolic activity in all cells is increased, and oxygenated blood reperfuses tissue beds. This is a particularly striking event for splanchnic organs that are typically hypoperfused compared with more anterior organs during torpor (36). Thus each time a hibernator arouses from deep torpor and resumes euthermic T_b (~37°C), it essentially shifts from a "cold storage" condition through a "warm reperfusion" period and then resumes a stable, healthy state that is typical for nonhibernators such as humans. There is evidence that hibernators are resistant to conditions that impair tissue perfusion and the reintroduction of blood flow to ischemic areas. In one study, hippocampal slices obtained from torpid and euthermic ground squirrels and euthermic rats were subject to aglycemic and hypoxic stress in vitro (91). Morphological damage induced by both stressors was greatest in brains from rats and lowest in brains from torpid squirrels, suggesting that the torpid state confers tolerance to conditions that simulate ischemic stress in vivo. Tolerance of hippocampal slices from active ground squirrels tended to be lower than torpid squirrels but greater than rats, particularly when tissues were tested at low temperature. Because the active squirrels were captured in autumn and presumably had already "prepared" for hibernation when killed, these results suggest that induction of stress tolerance may be

attainable in nonhibernators without a marked fall in T_b. Furthermore, increased tolerance was demonstrated early in a torpor bout (4 h), which also increases the likelihood that metabolic manipulation of nonhibernating species, such as humans, can be successfully employed on a short-term, transient basis. Other evidence that hibernation may confer a protective phenotype against ischemic injury comes from studies of $^{45}Ca^{2+}$ accumulation upon depolarization of brain synaptomes (101). Synaptosomes isolated from torpid ground squirrels exhibited reduced accumulation of $^{45}Ca^{2+}$ compared with active squirrels that were cold-adapted, an effect that appeared to involve a reduction in the activity of Q-type calcium channels (101). Because excessive Ca^{2+} entry has been implicated in tissue damage after ischemic injury, this effect may be an adaptation that provides neuroprotection for the hibernator during the greatly reduced cerebral blood flow that occurs during torpor. Hibernators may also offer new strategies for improved regulation of intracellular calcium concentrations during hypothermia and other stress conditions in the cardiovascular and other systems (250).

The reduction in metabolism that accompanies the fall in T_b as hibernators enter torpor is not only a function of thermodynamics, but also the result of precisely regulated metabolic reactions (128). Hibernating mammals survive the entire winter without feeding by limiting their carbohydrate catabolism and using fat stores as their primary source of fuel. Interestingly, the resulting increase in serum lipids that occurs immediately before and during hibernation (86, 96) may also provide ischemic-protective benefits (reviewed in Ref. 78). A critical question is whether ketone bodies are cytoprotective in brains of either hibernators or nonhibernators. Several lines of evidence suggest that both are the case. β-Hydroxybutyrate is beneficial in extending the survival of ground squirrels and mice subjected to acute hypoxia (73, 147). In these studies hypoxic tolerance was augmented when animals were ketonemic from either diet or acutely administrated β-hydroxybutyrate (82, 207). Unlike dietary protection, the hibernator releases a steady stream of free fatty acids from WAT. This increase in serum lipid probably fuels the massive 15-fold increase in circulating ketone body levels during hibernation (155). The known physiological benefits of ketone bodies may contribute to the resistance that hibernators display to conditions that normally induce whole body ischemia. Thus the continuous liberation of fatty acids during hibernation appears to mimic the benefits of the ketogenic diet.

B. Body Weight Regulation

Seasonal hibernators exhibit profound weight gain and fat deposition once each year as they prepare for hibernation. The ability to suddenly gain so much weight suggests that the animals become refractory to signals emitted from adipose tissue (e.g., leptin, Ref. 156) that normally contribute to body weight homeostasis by promoting fat deposition and then reducing further increases in fat stores as adipose tissue accumulates. Understanding the biochemical events in the hibernator that distinguish between the fattening and the homeostatic phases of the circannual rhythm will provide insights into the causes and control of obesity.

C. Use of Hypothermia in Surgery

Given the ease with which mammalian hibernators manipulate T_b as part of their physiological toolbox, it is logical to ask whether mechanisms underlying the use of regulated hypothermia by mammalian hibernators can be translated into improved survival of humans and other nonhibernating species in trauma situations. Indeed, it has long been recognized that hypothermia can provide protection against accidental or surgically induced trauma (143, 188). Mild whole body hypothermia can increase tissue tolerance to subsequent severe stress (187), and administration of hypothermia during reperfusion after cardiac arrest can significantly improve functional recovery (123). The mechanism(s) responsible for the protection afforded by hypothermia in trauma situations is only starting to be revealed, but upregulation of endogenous defense pathways such as stress proteins appears to be involved (123, 187). Further identification of the cellular and molecular mechanisms used by mammalian hibernators to transition into and out of hypothermic states may facilitate better use of hypothermia to reduce adverse outcomes for a variety of trauma situations.

D. Organ Preservation

Despite major advances in hypothermic preservation of organs before transplant, problems associated with extended cold storage and ischemia/reperfusion injury still limit the optimal use of transplantation as a therapeutic intervention for organ failure. Mammalian hibernators offer the unique opportunity to examine what is essentially nature's version of organ preservation. Surprisingly, few studies have actually tested whether hibernator organs remain viable for longer periods after in vitro cold storage than do organs from nonhibernating species, such as rats or humans. A preliminary report suggested that kidneys from hibernating ground squirrels are more tolerant to 3 days of hypothermic storage (as assessed by animal survival after organ transplantation) compared with kidneys taken from normothermic squirrels, rats, or rabbits (106). Recent studies at the University of Wisconsin (UW) compared the tolerance of livers from normo-

thermic rats and active and torpid ground squirrels to hypothermic storage in the UW solution (51). Rat livers when tested in an isolated perfusion system showed significant cellular damage as indicated by release of liver enzymes and depressed bile production after 48 h of cold storage. In contrast, livers from torpid ground squirrels showed minimal damage after 96 h of cold storage. This is significant, because it could be argued that organs of hibernators survive extended hypothermia in vivo because of the continuous (albeit greatly reduced) perfusion of tissues with oxygen and nutrients in the form of fatty acids. Demonstration that the hibernator liver is still superior to the rat when both undergo extended storage in vitro suggests that specific changes occur before hibernation that preserve organ viability even in the highly altered state of cold storage. Also encouraging from the standpoint of translation of hibernation-derived protective mechanisms to the clinical setting is the observation that damage to livers of nonhibernating (summer) ground squirrels after 72 h of cold storage is not as severe as occurs for rat livers but is still much greater than for torpid squirrels. Thus, within the same species, there is a switch from a less tolerant (summer) phenotype to a supertolerant (hibernating) phenotype. This suggests that systemic and/or cellular pathways that increase organ tolerance to cold ischemia/reperfusion are repeatedly induced within the same individual on an annual basis in association with the development of the hibernating phenotype. Preliminary studies indicate that greater tolerance of sinusoidal endothelial cells to cold storage/warm reperfusion may be one mechanism for enhanced tolerance in the torpid squirrel livers (52). Studies by other investigators using a different species, the Columbian ground squirrel, provided no evidence for superior maintenance of adenylate levels after several hours of cold storage of livers from torpid squirrels compared with euthermic squirrels or rats (62, 63). Elucidation of the mechanism(s) that underlie the increased tolerance to cold storage displayed by torpid hibernators, and strategies to adapt this information to organ preservation could have a profound effect on the quality, duration, and availability of human organs for transplantation. For example, a protein fraction with opioid-like qualities isolated from hibernating woodchucks suggested the use of δ-opioid agonists such as D-Ala^2-Leu^5-enkephalin (DADLE) in organ preservation (191, 216).

E. Muscle Disuse Atrophy

Hibernating mammals may provide a natural model system to study mechanisms that increase the tolerance of skeletal muscle to atrophy and dysfunction after extended periods of disuse. During hibernation, skeletal muscle mass in ground squirrels and bats is reduced by 14–65% depending on muscle type and species, yet unlike the effect of muscle disuse in nonhibernating species, specific activity of the oxidative enzyme citrate synthase is increased (225, 253). Black bears show no loss of skeletal muscle cell number or size from early to late in the hibernation season, and oxidative capacity is completely preserved or only slightly reduced (235). Muscle strength was reduced only 23% after 130 days of inactivity, which is much less than what would be predicted for humans under confined bed rest or simulated space flight conditions for a similar period (114).

IX. FUTURE DIRECTIONS

The ease with which hibernators survive their dramatic seasonal cycles of torpidity and arousal suggests that molecular, cellular, and higher-order mechanisms have evolved to successfully manage these events and permit survival during and after the hibernation season. The future translation of lessons learned from hibernators to biomedicine and other technologies requires a detailed understanding of differential gene expression between the active and torpid states. This understanding can only be achieved by a comprehensive approach that exploits advances in genomic and proteomic technologies to define the differential expression of mRNAs and proteins, and the posttranslational modifications of the proteins, that distinguish summer-active from winter-hibernating states as well as the various stages within the torpor-arousal cycle. This information is crucial for the development of rigorous and testable hypotheses that will provide a clear understanding of how mammalian hibernators achieve, survive, and reverse the remarkable physiological state of torpor. Furthermore, a detailed understanding of hibernation will pave the way for a variety of hypometabolic strategies that improve outcomes for human and animal health.

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