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NARRATIVE STUDENT REVIEW

Changes to Metabolism and Cell Physiology that Enable Mammalian Hibernation

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Abstract Heterothermy is a widespread, adaptive strategy used by many species of bird and mammal to conserve energy during periods of energetic deficit, the expression of which varies greatly depending on the species and environment. A temporary, reversible reduction in metabolic rate and body temperature (i.e., torpor) is an adaptive response used by many species of birds and mammals to conserve energy during periods of resource scarcity. Long-term employment of torpor (i.e., hibernation) is a seasonally expressed phenotype, the genetic and regulated pathways of which can be found throughout all mammal lineages, including hibernators and nonhibernators alike. In mammals, adaptations that allow for hibernation can be classified as those involved in preparation for hibernation, metabolic reduction, continued cellular function and protection, and arousal. Key physiological changes involve seasonal regulation of metabolic hormones, a shift to largely using endogenous fuel sources (i.e., increased lipolysis), global down regulation of protein transcription by posttranslational modification and microRNA save for the increased production of a small number of protective proteins, shifts in membrane composition, and thermogenesis by brown adipose tissue. There is some evidence of cold acclimations in nonhibernators, such as during fetal development, but responses are limited and cursory, and eventually cellular damage occurs. Therefore, it appears that a complete suite of adaptations to metabolism, vital physiological functions, and thermogenic mechanisms is required for the successful expression of the hibernation phenotype.

Keywords Cellular physiology · Gene expression · Hibernation · Hypothermia · Metabolism · Phenotypic plasticity · Torpor

Abbreviations

ATP	Adenosine triphosphate
BAT	Brown adipose tissue
MR	Metabolic rate
PUFA	Polyunsaturated fatty acid
T_a	Ambient temperature
T_b	Body temperature
T_{set}	Hypothalamic body temperature setpoint
WAT	White adipose tissue

Introduction

Endothermic animals rely on metabolically produced heat to maintain high body temperature (T_b) independent of environmental conditions [104]. Most enzyme-mediated reactions occur optimally within a very narrow temperature range [92], and endothermic thermoregulation ensures biological function over a wide range of ambient temperatures (T_a). However, there is considerable energetic demand for the maintenance of high metabolic rates. An adaptive thermoregulatory strategy known as torpor—the use of temporary, reversible reductions in metabolic rate (MR) and T_b—has evolved in some animals to deal with

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situations in which maintaining high T_b is energetically restrictive [77, 198]. Torpor is a physiological state of heterothermy in which MR and T_b are concomitantly and purposefully reduced to conserve energy, followed by an increase in metabolism and a rewarming of the body to a state of normal function using endogenous sources of heat [198].

Torpor can be defined more narrowly as being daily or seasonal (i.e., hibernation). Daily torpor is characterized by bouts lasting less than 24 h with T_b often remaining some degrees above T_a [176]. In contrast, hibernation is extended and often lasts for months with torpor bouts of multiple days to weeks interspersed with short periods of arousal and euthermy [124]. Hibernation can be further split into obligate hibernation, defined by predictably seasonal drops in MR, T_b, and activity regardless of external factors (e.g., [162]), and facultative hibernation when animals use torpor for extended (i.e., more than 24 h) periods outside the typical "hibernation season" in response to an ephemeral energetic challenge (e.g., [202]). During hibernation, body temperature setpoint (T_{set}) is considerably reduced and T_b often remains at or very near (within 1 °C of) T_a [85]—in the range of 2–10 °C for most hibernators, but as low as −2.9 °C in extreme cases [11, 139]. Concomitantly, the rates of biological processes are slowed to a fraction of normal values. Torpid metabolic rate during hibernation can be less than 1 % of resting metabolic rate [74], and heart rate can decrease by more than 97 %; for example, mean heart rate in the hibernating Gould's long-eared bats (*Nyctophilus gouldi*) drops to as low as 5 beats/min during hibernation compared to a mean of 228 beats/min in a normal resting state [49, 50]. Respiratory rate is also affected in torpor; it is not only reduced but also completely arrested for periods of time, with some hibernators experiencing prolonged periods of apnea [93].

Many small mammals and some birds use torpor. Examples of heterothermic species can be found throughout the classes Aves and Mammalia [77, 176, 198], including hummingbirds [127] and nightjars [27], marsupials [167], rodents [72, 150], bats [182], and primates [52]. The phylogenetically widespread distribution of heterothermic species (Fig. 1) suggests torpor is an ancestral trait [136]. Moreover, although there is considerable variation in the degree to which heterothermic species throughout the class Mammalia express torpor, species that are closely related tend to express similar torpor characteristics [176], which further suggests heterothermy is a plesiomorphic trait. With the exception of the common poorwill, *Phalaenoptilus nuttallii* [27], hibernation is restricted to mammals, mainly those species living in temperate regions that experience extended periods of food shortage (i.e., winter; [198]). The hibernation phenotype may have evolved in some lineages as an adaptation to further support maintenance and survival at lower T_b and slower MR during periods of energetic deficit [8, 112].

My review focuses on the cellular and physiological changes associated with hibernation, as well as the molecular underpinnings when appropriate. Adaptations associated with hibernation can be classified into four main categories (Fig. 2): (1) those that allow animals to prepare themselves for hibernation (i.e., put on fat); (2) those that result in state of metabolic reduction, and thus lower T_b; (3) those that allow continued cellular function in the cold and prevent damage induced by reduced biological synthesis; and (4) adaptations allowing for a rapid return to normal function upon arousal, which allows the oft-overlooked characteristic of hibernation in that it is regulated and all associated physiological changes are non-permanent and reversible. Arousal by endogenous means is a key feature for distinguishing torpor from unregulated hypothermia (i.e., uncontrolled periods of lower than normal T_b; [79]). The majority of our knowledge about hibernation physiology stems from research on rodents, particularly sciurid hibernators, such as ground squirrels and marmots. Seasonally obligate hibernators such as these represent the most extreme and most well-studied phenotype. Therefore, much of this review focuses on the hibernation biology of this group of mammals during the hibernation season. However, consistent patterns in metabolism and physiology can be found in all hibernators, including facultative hibernators that enter long-term torpor during other times of the year. In such cases, many changes are unnecessary or less pronounced, such as accumulation of large fat reserves, but the mechanisms of metabolic depression are likely the same as those used during seasonal hibernation.

Hibernation Triggers and Control of Physiological Processes

There is considerable debate as to which specific system triggers seasonal torpor and influences patterns of physiological activity once in hibernation. Activity is purported to be largely under the control of a master endogenous clock located within the suprachiasmatic nuclei (SCN) of the hypothalamus [173]. This system is driven by photoperiod and results in circadian (i.e., daily) oscillations in metabolism, appetite, and activity [124, 173]. Patterns of daily torpor appear to fall under circadian control, which would allow animals to continue to arouse and function (e.g., forage and locomote) during normal periods of activity, but circadian rhythm appears to have little influence on hibernation. Seasonal changes in day length and levels of light-driven hormones, such as melatonin, likely drive circannual rhythms as well. For example, waning daylight

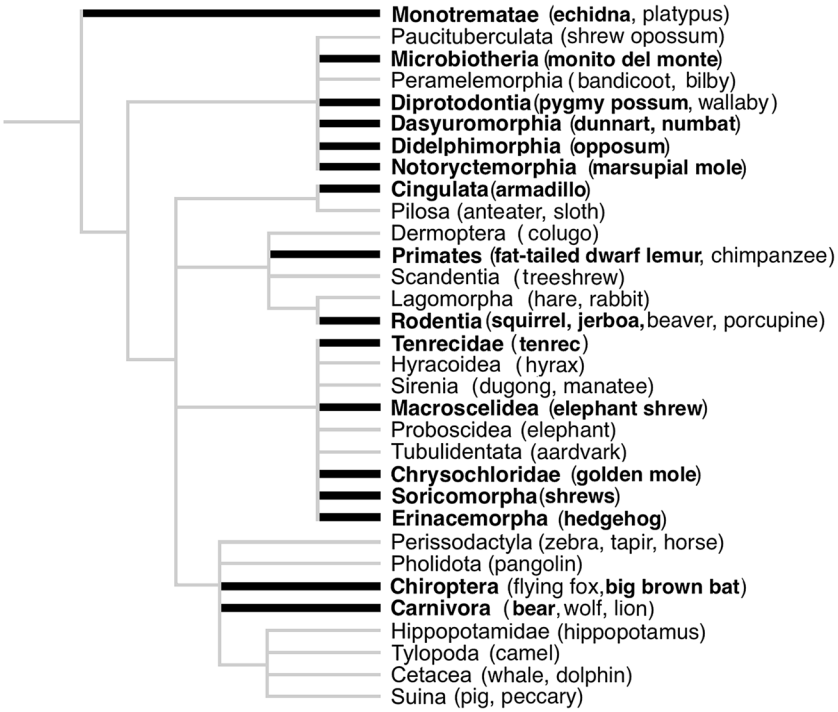

Fig. 1 Dendrogram depicting major groups (Families and Orders) within the class Mammalia that are known to use torpor (*black lines* and *bold text*). Examples of species within each group that use torpor appear in *bold text*. The wide distribution of torpor suggests it is an ancestral trait. The dendrogram was prepared using data from Lovegrove [136], Ruf and Geiser [176], and the Interactive Tree of Life [132]

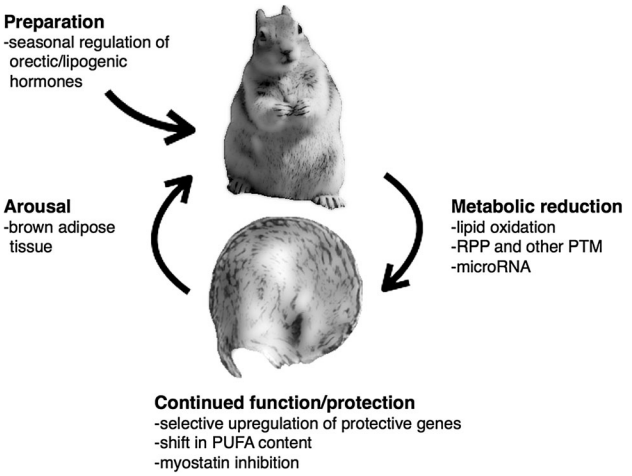

Fig. 2 A schematic outlining the four categories of adaptations for hibernation: (1) those that allow animals to prepare themselves for hibernation; (2) those that result in state of metabolic reduction and thus lower T_b; (3) those that allow continued cellular function in the cold and prevent damage incurred from reduced biological synthesis; and (4) adaptations allowing for a rapid return to normal function upon arousal

levels are purported to trigger seasonal pre-hibernation fattening [171, 172]. In addition, timing of seasonal hibernation is predictable (see Fig. 3) and in most cases occurs regardless of environmental conditions. Circannual rhythms of hibernation can persist even under constant photoperiod and temperatures (e.g., [53, 119]), which suggests intrinsic circannual control [124]. However, energy availability also influences entrance into hibernation. Reductions in food availability lead to metabolic stress [76], signaled by increased ratios of [ADP]:[ATP] and [NAD^+]:[NADH]. The cellular response to falling concentrations of energy substrates is a reduction in metabolic rate and an increased reliance on lipolysis for ATP production [76], two main characteristics of hibernation. Furthermore, phenology of hibernation is influenced by sex and body condition, and can vary within a species and even within a single population [201]. For example, male little brown bats (*Myotis lucifugus*) enter hibernation later than females and fatter females emerge earlier than other roostmates [157]. Thus, onset of hibernation appears to be an underlying response to photoperiod and influenced to some degree by other environmental factors that predictably wane prior to the hibernation season, such as the abundance of food on the landscape, as well as individual condition.

Photoperiod, however, is not likely to influence circadian rhythms of animals in concealed dens, roosts, or burrows, and food availability rarely fluctuates throughout the winter. This raises the question: what controls rhythms in activity and physiological processes during hibernation when these cycles are disrupted? Current opinion is split as to whether circadian control of activity during hibernation is modified to be photo-independent or completely abandoned. Transfer of control of physiological processes to an alternate, temperature-dependent circadian clock that is not affected by photoperiod would result in patterns of activity

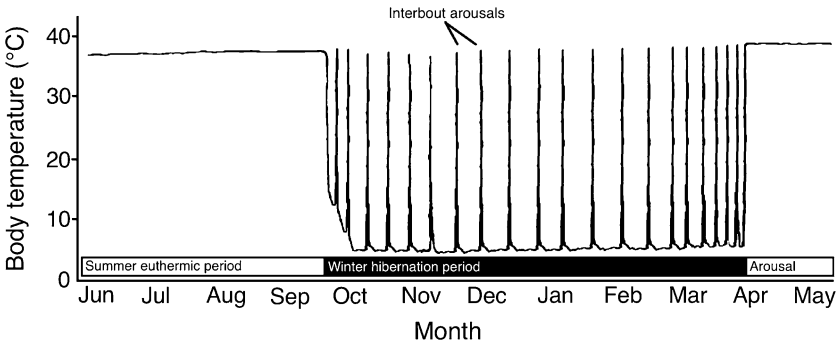

Fig. 3 Simplified schematic of T_b over time of a typical hibernator, showing euthermy in the summer, hibernation with frequent interbout arousals during the winter, and final arousal in the spring (T_b pattern adapted from Epperson and Martin [62])

that slow down with reduced T_b [141]. Under such control, maintenance of semi-regular patterns in arousal and activity (i.e., interbout arousals; see Fig. 3) during extended periods of darkness and quiescence would continue. The fact that duration of torpor bouts negatively correlates with T_b at temperatures above T_{set} during hibernation (e.g., [82]) seems consistent with this hypothesis. In addition, patterns of activity when arousals occur seem to remain under some circadian influence during part hibernation. For example, in hibernating Myotis bats (Natterer's bat, *M. nattereri*; Daubenton's bat, *M. daubentonii*; whiskered bat, *M. mystacinus*; and Brandt's bat, *M. brandtii*), timing of peak activity throughout winter remains linked to sunset and diurnal activity is uncommon [107]. However, there is evidence that the influence of a circadian rhythm is lost during hibernation. Clock genes *Period1* and *Period2* are expressed constantly during hibernation as opposed to a normal, rhythmic, diel pattern [165], and patterns of arousal during hibernation that contradict a diel rhythm have been reported. For example, arousals in some populations of little brown bats show no circadian pattern [51]. Furthermore, warmer ambient temperatures result in more frequent arousals by bats hibernating in buildings than in caves [89], which suggests roosting ecology (and therefore ambient conditions) affects arousal patterns. In general, currently available data are inconclusive as to whether a modified circadian clock persists or if another intrinsic timing mechanism is involved. Patterns of physiological activity are likely controlled by interactions between endogenous clocks (both circadian and circannual), photoperiod, temperature, resource availability, and metabolite imbalance.

The intrinsic mechanisms behind the onset of hibernation also remain poorly understood. Current opinion is that changes in MR and T_b during hibernation are controlled by way of a "sliding setpoint." Adaptations of the hypothalamus adjust the temperature around which T_b is metabolically defended [101]. Just as a furnace does not produce heat until T_a reaches the setpoint of the thermostat, metabolic heat production in the body is largely reduced until T_b reaches the newly established setpoint (T_{set}). During entrance into hibernation, decreased T_{set} and concomitant reduction of metabolic rate precede a more gradual decline in T_b [102]. The metabolic response of hibernating animals when T_b drops below T_{set} is also important and further supports the idea of setpoints rather than complete abandonment of thermoregulation. As T_b drops below T_{set}, metabolic activity increases proportionately and torpor bouts are shortened to bring T_b back up to T_{set} (e.g., [56, 78]). This further suggests thermoregulation during hibernation is not completely abandoned and hypothalamic control is maintained at low temperatures [101]. Ecologically, maintenance of T_b around a setpoint lends importance to hibernacula microclimate; T_a in hibernacula above or below the species-specific T_{set} increases metabolic activity and diminished energy savings.

Although the precise mechanisms involved in hypothalamic control of T_{set} and T_b remain largely unknown, recent evidence suggests involvement of central A1 adenosine receptors (A1AR) in the preoptic area of the hypothalamus (POAH) and the circadian-regulated nucleotide adenosine 5′-monophosphate (AMP). Neurons within the POAH monitor T_b and regulate appropriate responses [149]. Activation of A1AR appears necessary for torpor; blocking A1AR activity prevents and disrupts hibernation [113] and A1AR agonists can successfully induce a torpor-like state, even in nonhibernating lab rats (*Rattus norvegicus* [193]). In addition to activating A1AR in the POAH, AMP also reduces activity of neurons responsible for inducing a thermogenic response to cold, which ultimately lowers T_{set} [153]. Levels of AMP are elevated in the blood of hibernating mice kept in constant darkness, and AMP induces hypothermia in mice (*Mus* spp.) despite normal circadian light cycles [212]. Seasonality of hibernation also suggests a relationship between AMP and A1AR in inducing hibernation. Stimulation of A1AR induces metabolic suppression during but not outside of the hibernation season (i.e., winter) in Arctic ground squirrels (*Urocitellus parryii* [116]), likely due to seasonal adenosine sensitization (i.e., increased A1AR density) in the winter or relatively low levels of AMP during summer. These findings support the role of AMP and its effect on A1AR in the onset of torpor.

However, physiological parameters measured during induced hypothermic states are typically inconsistent with those of true hibernation [186, 187].

Adaptations in Preparation of Hibernation

Fat Storage

The most outwardly obvious change in preparation for hibernation is the accumulation of large fat stores (e.g., [17, 31, 128]). Food intake doubles or triples preceding hibernation, and body mass increases by as much as 62 % in free-ranging individuals [177]. In nonhibernators, satiety signals maintain body adiposity and energy balance. Numerous pathways and proteins activated by accumulation of surplus adipose tissue elicit a counter response. For example, adipocytes in white adipose tissue (WAT) release leptin when adiposity is high, which acts on the hypothalamus in long-term suppression of appetite and enhances lipid oxidation [35, 183]. Leptin also inhibits AMPK-activated protein kinase (AMPK), a metabolic "master switch" that stimulates appetite [123] and ATP production from energy stores [147]. High adiposity also reduces hunger by inhibiting the release of ghrelin, a hormone that stimulates appetite and food intake [48]. Physiological changes in hibernators allow them to temporarily override signals of adiposity to continue pre-hibernation fat storage.

Concentrations of leptin and ghrelin typically follow seasonal fluctuations (reviewed in [96]). Body mass and leptin levels continue to increase concomitantly during the pre-hibernation period in woodchucks (*Marmota monax* [45]), and leptin is positively correlated with increasing fat mass and fat cell size during pre-hibernation mass gain in yellow-bellied marmots (*Marmota flaviventris* [67]). An increase in leptin levels is expected with hyperphagia, but its anorexigenic (i.e., appetite suppressing) effects are inhibited, suggesting seasonal resistance. In little brown bats, a leptin increase occurs just prior to fattening but decreases during fattening, suggesting complete dissociation of leptin release from adiposity during pre-hibernation fattening [126]. Appetite can be suppressed with a leptin injection during pre-hibernation hyperphagia in Arctic ground squirrels, which also suggests sensitivity to the hormone is retained but dissociation occurs [160]. Regardless of mechanism, seasonal regulation of the anorexigenic effect of leptin allows hibernators to continue to eat and fatten during pre-hibernation. Leptin sensitivity is restored shortly after entering hibernation; high concentrations of leptin in hibernating Columbian ground squirrels (*Urocitellus columbianus*) continued to restrict food intake despite food being available [68]. Likewise, golden-mantled ground squirrels (*Callospermophilus lateralis*) experimentally induced to hibernate in warm temperatures ($T_a = 22$ °C) had higher metabolic rates, declining fat reserves, and an associated drop in leptin levels and return of appetite [68].

Ghrelin inhibition also appears to be seasonally regulated in hibernators. Despite increasing adiposity during the hyperphagic pre-hibernation period of golden-mantled ground squirrels, levels of ghrelin remain higher than any other time of the year [99], suggesting a seasonal upregulation of circulating levels of the hormone [192, 209] and possible dissociation from the adiposity feedback loop. In addition to directly stimulating appetite, ghrelin amplifies its hyperphagic effect by phosphorylating and activating AMPK [97, 98]. Although AMPK stimulates appetite, it also inhibits lipogenesis and other anabolic processes that are counterproductive during pre-hibernation fattening [90]. However, anabolic-inhibiting effects of AMPK are not observed in the fall. Seasonal dissociation of AMPK may mitigate this conflict, but a test of this idea is required.

Differential insulin sensitivity also plays an important role in pre-hibernation adiposity. Plasma insulin promotes uptake of blood glucose to be stored as glycogen in the liver and muscles, decreases lipolysis and increases lipid synthesis, and stimulates the production of triglycerides and adiposomes [208]. Effects of plasma insulin are therefore advantageous during periods of pre-hibernation fat accumulation. However, levels of plasma insulin are proportional to levels of cerebrospinal fluid (CSF) insulin, which decreases appetite and thus inhibits adiposity [207]. The responses of hibernating animals to insulin suggest a seasonal sensitivity. In both golden-mantled ground squirrels and yellow-bellied marmots (*Marmota flaviventris*), insulin level increases expectedly with adiposity during autumn fattening [21, 66] but does not signal satiety [66]. In grizzly bears (*Ursus arctos horribilis*), insulin sensitivity also follows seasonal patterns with adipocytes showing three distinct levels of responsiveness throughout the cycle [155]. During pre-hibernation fattening, adipocytes are hypersensitive to serum insulin only to become insulin resistant during the onset of hibernation and then resume normal levels of insulin sensitivity post-hibernation [155]. Selective insulin resistance appears similar to leptin resistance in that it is reversed shortly after the individual enters hibernation [65]. Insulin levels remain low throughout much of the hibernation period in most species [65] but gradually rise during the last half of the hibernation period in the little brown bat [14]. This may reflect the importance of a readily available energy pool immediately upon arousal and emergence to ensure the ability to fly.

Although the exact mechanism behind selective hormone resistance is unclear, a recent study hints at tissue-specific changes to hormone receptors as having a role. Regardless of age or sex, androgen receptors of pre-hibernating Arctic ground squirrels are selectively upregulated in skeletal and brain tissue [19]. This stimulates building of muscle tissue, which is necessarily catabolized along with lipids during hibernation in the harsh Arctic climate, without costs typically associated with high testosterone levels in other systems, such as immunosuppression [135] or increased reproductive effort [204]. Whether selective receptor regulation is responsible for the shifts in sensitivity to other hormones, such as insulin, during hibernation remains to be seen but is a promising place to start.

Metabolic Adaptations for Hibernation

Metabolic Fuel

The primary source of fuel for mitochondrial respiration shifts from carbohydrates to lipids during hibernation. Indirect evidence of reliance on lipids as a fuel source comes from the observation of a reduction of fat stores as winter progresses (e.g., [21, 31]). Measurable shifts in respiratory quotient (RQ; CO_2:O_2 in exhaled breath) also indicate a switch from carbohydrates to fats during hibernation. A RQ value close to 1.0 indicates carbohydrates are the main metabolic substrate, whereas values of 0.7 suggest lipid oxidation [138]. During hibernation, RQ in European hedgehogs (*Erinaceus europaeus*) is 0.7, indicating lipid metabolism [188]. Likewise, in Arctic ground squirrels, RQ averages 0.70 during steady-state torpor at T_a between 4 and 8 °C, indicating exclusive use of lipids for energy production [32]. However, outside this range of temperatures at −16 and 20 °C, RQ rises to values >0.85, suggesting a return to primarily carbohydrate-fueled metabolism during arousal [32].

Some mechanisms behind the switch in metabolic fuel source simultaneously inhibit the use of carbohydrates and increase the availability of fatty acids. Upregulation of genes for pyruvate dehydrogenase kinase isoenzyme 4 (PDK4) and pancreatic triacylglycerol lipase (PTL) occurs in the heart, skeletal muscle, and WAT of thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) during hibernation [7]. Induction of PDK4 inhibits activity of pyruvate dehydrogenase (PDH) by phosphorylation. Conversion of pyruvate to acetyl-CoA is dependent on PDH, and inhibition of this pathway halts entry of glycolytic intermediates into the TCA cycle [6]. In conjunction with PDK4 inhibition of glycolysis, PTL stimulates lipolysis by breaking down adiposomes (i.e., "fat droplets") to release fatty acids for use in the β-oxidation pathway. High lipolytic activity at low temperature is inherent to PTL and conserved across all mammal lineages, but its upregulation and consequential surge during lipolysis appears to be unique to the hibernating state [5]. Other proteins that are elevated during hibernation include liver fatty acid-binding protein, fatty acid transporter, and 3-hydroxy-3-methylglutaryl-CoA synthase, all of which support the switch to lipid metabolism [63].

Metabolic Rate

In mammals, 90 % of oxygen consumption occurs in the mitochondria, of which 80 % is involved in ATP synthesis and 20 % in maintaining ion concentration gradients [169]. Given that mitochondrial respiration is a substantial site of energy turnover and heat production, changes within are key to metabolic reduction and T_b reduction. Rates of enzymatic reactions decrease as temperature drops [92]. The temperature coefficient of metabolic activity (Q_{10}) is the change in rate of oxygen consumption over a 10 °C temperature range [91]. Most biological reactions have a Q_{10} of 2–3, but values >3 are typical during hibernation, suggesting additional physiological changes to inhibit metabolic activity beyond that expected based on decreases in T_b alone [74]. As much as 76 % of metabolic depression in hibernating edible dormice (*Glis glis*) is purportedly due to physiological inhibition as opposed to thermodynamic effects [100].

Decreases in metabolic rate are correlated with reductions in enzyme activity and available substrate [169]. For example, activity of glycogen phosphorylase, which converts stored glycogen into glucose, decreased by as much as 75 % of euthermic levels in liver and kidney of golden-mantled ground squirrels [29]. Reversible protein phosphorylation (RPP) has emerged as a crucial mechanism in the shift between active and inactive states in both metabolic enzymes and substrates [184]. Substantial reduction in metabolic activity occurs when metabolic enzymes and substrates are inhibited by covalent addition of phosphate groups, a potentially widespread process carried out by protein kinases and quickly reversed by protein phosphatases [184]. In golden-mantled ground squirrels, the proportion of active pyruvate dehydrogenase (PDH), which converts pyruvate into carbohydrates that enter the tricarboxylic acid (TCA) cycle, drops from 60 to 80 % in heart and kidney of euthermic animals to as little as 4 % during hibernation, the rest inactivated by RPP [29]. Phosphorylation of skeletal muscle hexokinase (HK), an enzyme that catalyzes the conversion of hexoses into hexophosphate, also occurs during hibernation [2]. In Richardson's ground squirrels, activity of phosphorylating HK is reduced by 33 % compared to euthermic values [2].

This is particularly important for the reduction of glycolysis because HK converts glucose to glucose-6-phosphate (G-6-P), which then enters cells and is used in glycolysis. Deactivating enzymes involved in carbohydrate catabolism by RPP facilitates the switch from carbohydrates to lipids as the main metabolic fuel source and is rapidly reversible [184].

Most cellular processes that consume energy are directly or indirectly associated with membranes and the maintenance of concentration gradients [169]. Thus, activity of membrane-associated proteins has profound effects on metabolic rate [61, 110] and regulated suppression of ion channels by RPP is a key factor in reducing ATP turnover and metabolic reduction. For example, phosphorylation of Na^+/K^+-ATPase (i.e., sodium–potassium pump) in golden-mantled ground squirrels results in reduction of that pump's activity by up to 60 % [185]. Likewise, RPP of sarcoplasmic reticulum Ca^{2+}-ATPase in hibernating long-tailed ground squirrels (*Urocitellus undulates*) reduces activity of the pump by 50 % compared to active animals [142].

Protein synthesis is responsible for up to 10 % of resting metabolic rate in mammals [169] and is largely globally downregulated during hibernation [122]. For example, in hibernating thirteen-lined ground squirrels, the rate of protein synthesis is reduced to 34 % of the euthermic value in brain tissue [73] and 15 % in kidney tissue [106]. Likewise, in torpid golden-mantled ground squirrels, transcription is reduced to 50 % of the value in euthermic animals between bouts of torpor [195]. Observed decreases in activity have Q_{10} values between 2 and 3, which are to be expected based on temperature effects alone [195]. However, at least some suppression of gene expression is purportedly due to posttranslational modification of proteins [185]. Small ubiquitin-related modifiers (SUMOs) have been shown to target and turn off transcription factors [86]. The brains, liver, and kidney of hibernating thirteen-lined ground squirrels showed elevated SUMOylation of proteins [131]. The effect of SUMOylation is reproducible in human-derived research cells, suggesting the underlying mechanism is not unique to hibernators [131]. However, the large-scale use of such modifications in metabolic reduction is not evident outside mammals with the hibernation phenotype.

Interestingly, a number of key metabolic proteins seem largely absent in metabolic rate depression during hibernation. In particular, AMPK has a central role in regulating energy balance in euthermic states and could be expected to have an essential role in global metabolic depression. AMPK is activated by a rise in cellular ADP:ATP ratio and stimulates catabolic ATP-generating processes, including mitochondrial biogenesis [166]. However, most studies report only tissue-specific changes in AMPK levels during hibernation. In thirteen-lined ground squirrels, relative abundance of AMPK was three times higher in WAT of winter animals compared with that of summer animals, but there were no differences in other tissues [108]. Similarly in golden-mantled ground squirrels, only WAT showed higher total AMPK abundance in hibernation compared to euthermy, with no differences detected in brown adipose, muscle, or liver tissue [98]. The fact that changes in AMPK expression are not global and occur only in WAT suggests AMPK does not play a key role in metabolic depression during hibernation. Additional regulation of AMPK may not be necessary given that disruption of ADP:ATP ratio might occur during entry into torpor [118] but is largely restored once ATP-genesis and ATP-turnover balance in steady-state hibernation. Metabolic fuel source shifts from carbohydrates to lipids during hibernation, and higher AMPK levels in WAT may conserve metabolic ability of these tissues despite low T_b.

Key Adaptations to Cold and Quiescence

Gene Expression

Steady-state levels of most mRNAs and proteins do not decrease considerably during hibernation, despite reduced synthesis [39, 62, 180, 181]. Enzyme kinetics predicts protein degradation to slow down as T_b drops, but protein loss and degradation of mRNA proceed even slower than expected given the length of time that hibernators remain dormant. Mechanisms exist to conserve genetic precursors during hibernation, such as a seasonal increase in the robustness of the poly(A) tail of mRNA [122]. Residues along the poly(A) tail of mRNA isolated from hibernating Arctic ground squirrels showed increased binding sites for stabilizing proteins, an alteration not seen in summer-active ground squirrels [122]. Expression of proteins involved in folding, stabilizing, and transporting other proteins is also increased [63]. Nevertheless, protein loss does occur and may be a reason for interbout arousals. Protein synthesis during arousals is rapidly increased and often exceeds the rate during the active season [63].

A small number of some genes are upregulated during hibernation, aside from those associated with metabolism already mentioned (e.g., PDK4 and PTL). Levels of some proteins produced in the liver and released into the blood are increased to preserve cellular function at low temperatures, such as α2-macroglobulin (α2m; [62, 180]), which prevents clotting and aids in blood perfusion at low temperatures [181], and apolipoprotein A1 (apoA1), which transfers long-chain fatty acids to target tissues [62]. Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) is upregulated in the heart of hibernating woodchucks relative

to active conspecifics [25]. Increased activity of SERCA not only preserves cardiac function at low T_b by maintaining Ca^{2+} balance [87], but also provides localized metabolic heat within the membrane of cardiomyocytes [54]. Selective regulation of protein synthesis allows hibernators to decrease energy consumption while simultaneously maintaining levels of those proteins important for cellular function.

MicroRNA (miRNA), small non-coding transcripts that bind to mRNAs and target them for degradation or inhibit their translation [13], has recently been described as having a pivotal role in controlling global gene expression during hibernation. Studies investigating miRNA expression during hibernation are limited but promising. Selective and tissue-specific regulation of miRNAs may preserve critical organ function and contribute to metabolic change during hibernation. In thirteen-lined ground squirrels, levels of miRNA-1 and miRNA-21 in kidneys are 100 and 30 % higher in hibernation compared to euthermy [148]. Increased cell proliferation is associated with miRNA-1 expression [43], and miRNA-21 inhibits cellular apoptosis [41]. Maintenance of kidney structure and function during hibernation is likely aided by upregulation of these miRNAs. Other miRNAs are downregulated during hibernation to reduce ATP turnover and promote lypolysis. Levels of miRNA-24 and miRNA-122a transcripts are reduced in heart and skeletal muscle of torpid animals [148]. Reduction in miRNA-24 activity is implicated in the suppression of cell growth and differentiation [129], and inhibition of miRNA-122a reduces lipogenesis and promotes fatty acid oxidation [64]. The role of miRNAs has only recently been suggested in hypometabolism, and empirical data are sparse. Given that microRNAs are evolutionary conserved in all animals and regulate 30–50 % of all protein-coding genes [13], their involvement in hypometabolism is highly likely and further research is warranted.

Membrane Function

Lipid membranes are selectively permeable boundaries vital to almost all cellular processes. Membrane fluidity and activity of inter-membrane pumps are reduced as temperature drops [4, 42]. Cold-induced leakage and disruption of ion gradients typically causes catastrophic loss of cellular and organ function [33, 199]. Unlike the widespread polyunsaturation observed in cold-adapted ectotherms [46, 95], a phenomenon known as "homeoviscous adaptation" [178], mammalian hibernators maintain membrane function at cold temperatures without consistent patterns of polyunsaturation, suggesting different mechanisms are involved [4].

Most membranes show some change in unsaturation index during hibernation, and there are measurable effects on hibernation. Increased consumption of dietary PUFAs in the fall facilitates entrance into torpor and lengthens and deepens torpor bouts during hibernation (e.g., [69, 75, 81]). However, increased membrane PUFA composition is a double-edged sword; a global increase in total membrane PUFA concentration would result in a concomitant and counterproductive rise in membrane-associated metabolic activity [109]. PUFAs are also more prone to react with reactive oxygen species (ROS), which causes peroxidative damage [70], making high concentrations unfavorable. Animals experimentally fed diets higher in PUFA than would be normally ingested hibernate at higher T_b and for shorter bouts than those fed moderate levels of PUFA (e.g., [70]). Rather than an increase in total membrane PUFA content, a shift in the ratio of n-3:n-6 class PUFAs is central in maintaining membrane function at low temperature. A higher proportion of n-6 fatty acids (e.g., linoleic acid) to n-3 fatty acids increases membrane-bound pump activity [175] without increasing the negative effects associated with PUFA. Hibernators also employ tissue-specific shifts in membrane PUFA composition, and increased total PUFA levels mainly occur in adipose tissue [151]. Oxidative stress caused by increased PUFA levels may additionally be offset by the inherently lower production of peroxides during ATP synthesis in some hibernators. For example, the mitochondria of little brown bats produce as little as one-third the amount of hydrogen peroxide per unit oxygen consumed during respiration than those of short-tailed shrews (*Blarina brevicauda*) and white-footed mice (*Peromyscus leucopus*; [30]).

Cholesterol also increases membrane fluidity at low temperature [179] but there is a distinct lack of evidence compared to studies on PUFA to suggest an active increase in cholesterol uptake by free-ranging hibernators. There is an increase in total cholesterols (plasma, HDL, and LDL) during hibernation, but this appears to be related to increased lipid metabolism in a closed system rather than functional membrane change [161]. However, increased dietary cholesterol has been shown to influence torpor patterns in experimentally hibernated animals, resulting in longer torpor bouts at lower T_b [83]. Increased cholesterol concentration would allow for increased fluidity and function of cell membranes at lower temperatures. It could be that tissue-specific shifts in cholesterol concentration occur during hibernation as is seen with PUFA, but to my knowledge this has not been observed.

There are also inherent differences in pump substrate affinity and configuration that enables hibernators to maintain optimal rates of ion turnover at lower temperatures than nonhibernators. For example, activity of erythrocyte Na^+/K^+ pumps (i.e., Na^+/K^+-ATPase) in Columbian ground squirrels and guinea pigs (*Cavia porcellus*) is similar at high temperatures (37 °C), but turnover

rates are 2–3 times higher in ground squirrels at 5 °C [60]. Affinity of Na^+/K^+-ATPase for ATP is reduced at low temperatures in cold-sensitive cells (e.g., those of guinea pigs), whereas cells of hibernators show an increase in ATP affinity of Na^+/K^+-ATPase when cooled [143]. Cells of hibernators also maintain calcium balance better than nonhibernators at low temperatures. Rats exhibit an increase in the influx of Ca^{2+} into the cytoplasm as temperature drops, while the opposite is true in Columbian ground squirrels because fewer calcium channels are present and open [134]. Likewise, Richardson's ground squirrels have fewer Ca^{2+}-ATPase and higher threshold activation levels in smooth muscle compared to guinea pigs [205]. Having fewer, less responsive ion channels allows for greater control over ion flux at cold temperatures, but there are associated costs. Stronger stimuli are likely needed for muscle contraction; thus, performance may suffer even at euthermic temperatures. Such trade-offs are consistent with the hypothesis that hibernators are thermal generalists and perform well over a wide range of temperatures but are not thermal specialists (i.e., obligate homeotherms) within their optimum temperature range (i.e., at euthermic temperatures; [8]).

Neural Function

Continued function of the nervous system is critical during hibernation. Cardiovascular performance needs to be regulated, and animals need to remain responsive to external stimuli. Although T_{set} varies depending on species and environment, hibernators typically avoid reaching potentially damaging T_b much below freezing and actively increase metabolic rate and the use of non-lipid fuels to do so [32]. Bats arouse even with non-tactile stimuli [190], suggesting hibernating animals remain attuned and responsive to their environment. Furthermore, the continued ability to regulate vital processes while in hibernation is not the only neural function conserved. Although previous studies on the effects of hibernation on memory retention have shown conflicting results (e.g., [144–146]), more recent studies tend to show no evidence of adverse effects. For example, Alpine marmots (*Marmota marmot*) trained to perform tasks before hibernating showed no decrease in their ability to complete these tasks after a hibernation period of six months [44], and greater mouse-eared bats (*Myotis myotis*) trained to find food in a maze performed at the same level pre- and post-hibernation [174]. An inherent benefit of quiescence may be that the lack of stimuli reduces the formation of new neural networks and junctions and increases the ability to maintain current pathways and synaptic associations. However, without mechanisms to protect and preserve these pre-hibernation memories, they would be expected to diminish to some degree during hibernation.

Bouts of cerebral ischemia result in a permanent loss of dendritic branching and synaptic connections, as well as permanent neuronal damage or death [24]. Neural function does decrease during long bouts of hibernation, but neurons of hibernating animals show reduced temperature-dependent loss of synaptic associations and remarkable plasticity. Hypothermia contributes inherently to decreased damage from ischemia. Ischemia-induced cell death primarily occurs by excitotoxicity, which is damage to neurons from massive efflux of neurotransmitters and excessive excitation, ultimately caused by inability to maintain cellular concentration gradients [211]. Cold temperatures inhibit the release of neurotransmitters, such as glutamate, thereby decreasing the risk of excitotoxicity [10]. However, there is a further and prolonged decrease in the activity of ion channels during hibernation than would be expected based on temperature alone.

Channel arrest and downregulation of neuroreceptor activity further protects neurons during hibernation. Actively downregulating *N*-methyl-D-aspartate receptors (NMDAR; a predominant glutamate receptor) by dephosphorylation during hibernation decreases the risk of excitotoxicity [170]. Hibernating Arctic ground squirrels show decreased phosphorylation and function of NMDAR compared to conspecifics during arousals and rats [213]. Reduction of nerve branching does persist during hibernation but may be an adaptation to further lessen the risk of excitotoxicity. Limiting the connectivity between neurons reduces the potential for a glutamatergic surge upon arousal [140].

However, mechanisms to protect neural function in hibernators are not perfect. Although slowed, decreases in neuronal function do occur during long, deep bouts of hibernation as a result of decreased electrical activity. Sleep, particularly rapid eye movement (REM) sleep, is necessary for refreshing somatic connections and maintenance of memory [20]. These restorative functions of sleep do not occur at T_b below 25 °C during hibernation [125, 196], and the neural effects of torpor are similar to those seen after periods of sleep deprivation [55]. Hibernating animals may therefore arouse periodically to "sleep" and restore neural function. Dendrites of ground squirrels reduced during the course of hibernation are rapidly regenerated upon arousal with no permanent damage [163]. European hamster (*Cricetus cricetus*) also show decreases in the length and complexity of branching of neurons in the intrahippocampal region during hibernation, but there is a remarkable and total reversal to pre-hibernation profiles within 2 h following arousal [140].

Cardiovascular Function

Cold-induced influx of Ca^{2+} into the cytosol causes arrhythmias in cold-sensitive cells [33, 199]. Hibernators maintain calcium balance much better than nonhibernators. European hedgehogs and Richardson's ground squirrels display a distinct lack of intracellular build-up of Ca^{2+} with falling temperature, while guinea pigs show a predictable linear increase in cytosolic Ca^{2+} concentration [88]. This is partly due to fewer, less responsive membrane-bound Ca^{2+} channels in hibernators but tissue-specific membrane polyunsaturation also plays a role. The lack of unsaturation in plasma membranes during hibernation is not global. Higher levels of PUFA occur in the sarcoplasmic reticulum of cardiomyocytes and increase SERCA2a activity [87]. Sequestration of Ca^{2+} in the SR by PUFA-facilitated SERCA2a activity and a concomitant shift toward n6-class PUFA in the cell membrane (as seen in other tissues) result in less cytoplasmic Ca^{2+} and proper functioning of the heart even at low T_b [200].

Skeletal Muscle Function

Maintenance of muscle tissue during hibernation is crucial for shivering thermogenesis and locomotion during arousals and upon emergence. In most mammals, periods of inactivity and disuse lead to breakdown of muscle vasculature [194] and muscle atrophy [168, 191]. However, atrophy is minimal, often nonexistent, in the muscles of hibernators [47, 130, 210]. For example, the muscle fiber properties of flight muscles in hibernating little brown bats and big brown bats (*Eptesicus fuscus*) under natural conditions are the same pre- and post-hibernation [26, 103]. This is unexpected for such long periods of dormancy, especially when compared to rapid and considerable muscle loss from starvation [15, 16], disuse [191], or unloading [152, 168].

Maintenance of muscle tissue during hibernation is due to a combination of adaptations that inhibit muscle protein loss. Inactivity and starvation typically stimulate expression of myostatin, a protein that inhibits growth and differentiation of muscle tissue, causing muscle atrophy [115]. However, assays of muscle tissue sampled from thirteen-lined ground squirrels during different periods throughout hibernation showed no increase of myostatin while entering or during hibernation [28]. The mechanism behind the downregulation of myostatin is unclear, but that myostatin levels increase markedly early during arousals suggests physiological inhibition. The protein p38 mitogen-activated protein kinase ($p38^{MAPK}$) may also play a role in retention of muscle composition. In hibernating little brown bats, $p38^{MAPK}$ and several downstream proteins are phosphorylated (i.e., activated) and in higher abundance compared to euthermic conspecifics [59]. Effects of activating the $p38^{MAPK}$ pathway include enhancement of lipid catabolism and prevention of muscle atrophy [59]. Interestingly, muscle mass may even increase despite continued inactivity during hibernation in some species. In thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*), protein synthesis in skeletal muscle drops 66 % from summer levels and muscle volume declines proportionally to other tissues with declining body mass during early hibernation (October through February). However, the ratio of muscle volume to body mass shows a disproportionate increase in the months prior to emergence in May [105]. The fact that no other tissues examined (heart, liver, and intestine) showed such increases suggests muscle tissue is not only maintained but also prioritized and actively synthesized mid-hibernation [105]. The mechanism behind this shift is unclear but could involve seasonal, tissue-specific androgen sensitivity, which has only recently been documented in Arctic ground squirrels [19].

RPP plays a role in maintaining muscle function in cold, anaerobic states. The enzyme creatine kinase (CK) uses ATP to catalyze the conversion of creatine (Cr) into phosphocreatine (PCr), which acts as a readily available source of phosphate for the later conversion of ADP to ATP under anaerobic conditions in muscle tissue [197]. In hibernating Richardson's ground, CK mRNA is reduced by 70 % compared to euthermic values, but CK activity and protein are reduced by only 20 % [1]. The balance occurs as a pool of available but phosphorylated (i.e., inactive) CK [1]. Phosphorylation of CK is quickly reversed, and the conversion of Cr into PCr to power muscle activity is rapidly resumed during arousal [1]. Similarly, hexokinase (HK) is differentially phosphorylated between hibernation and euthermy [2]. Hexokinase facilitates glycolysis by phosphorylation of glucose into G-6-P, the main sugar used for glycolysis [2]. In Richardson's ground squirrels, HK persists in low and high phosphate forms during hibernation, but is quickly and completely phosphorylated (i.e., activated) by AMPK during arousal [2]. Thus, in hibernators, muscle metabolism and activity is mediated through regulated and reversible phosphorylation of key enzymes. The lag between ramping up protein and energy substrate production during arousal is mitigated by dephosphorylating an existing pool of enzymes. The benefit is an immediate source of energy for shivering thermogenesis and movement upon arousal.

Other Adaptations

Immunosuppression is a consequence of low T_b [22, 34]. Entrance into hibernation is associated with a temperature-dependent reduction of plasma leukocytes, with as many as

90 % sequestered in lymphatic organs [23]. Reduction in immune capacity leaves hibernating individuals susceptible to parasites and disease, but it also prevents the wide-scale recruitment of cytokines and consequent inflammatory response that typically follows cerebral ischemia [3, 159]. Immune responses, such as fever, are absent during hibernation but common immediately or shortly after individuals arouse [164].

Appetite is suppressed during hibernation, and in the absence of eating, the gut undergoes some reduction in mass. For example, in Alpine marmots, stomach and small intestine mass is 105 and 259 % greater during mid-summer than the end of hibernation, respectively [111]. In addition, no net flux of nutrients occurs in mucosal tissue during hibernation [37, 40]. However, despite mucosal atrophy and reduced mass of gastrointestinal organs during hibernation, absorptive capacity is retained. Intestinal tissue of hibernating thirteen-lined ground squirrels showed no net nutrient absorption at hibernation T_b (7 °C), but when warmed to euthermic T_b (37 °C), absorption was highest in tissue of fall and winter animals [37]. High capacity for absorption is expected during fall hyperphagia but retention throughout winter is not expected given the cost of the required protein synthesis. By sustaining high absorption capacity in less tissue, hibernators save energy on tissue maintenance and conserve the ability to resume digestive function when critical, such as during arousals (if the hibernator caches food and eats periodically during winter) and at the end of hibernation when body mass may be low and body condition affects other life history traits, such as reproduction (perhaps especially important for insectivorous hibernators that rely completely on fat reserves). Retention of absorptive capacity may be due to persistent expression of moesin in hibernating animals. Moesin is associated with formation of microvilli in the intestinal brush-border membranes of hibernating mammals, but is not found in euthermic animals or in adult nonhibernating mammals [38].

Requirements for Rewarming and Arousal

Rewarming using endogenously generated heat is characteristic of torpor [114], and all small mammals, aside from tenrecs (*Tenrec ecaudatus* [137]) periodically arouse throughout hibernation [71, 203]. Restoration of neural function [163], mating [12], maintenance of muscle tissue [210], and access to food and water [112] have all been suggested as reasons for arousal. For whatever the reason, arousals play an essential role in hibernation biology and use a disproportionately large amount of energy [9, 56]. Over 80 % of energy reserves can be spent warming up and maintaining high T_b during arousals and euthermic bouts [76]. Thus, it is essential that hibernators have mechanisms to make this process as efficient as possible. One such adaptation is the extensive use of brown adipose tissue (BAT) for rewarming.

Non-shivering Thermogenesis

Non-shivering thermogenesis is largely dependent on BAT, which provides the predominant source of energy and heat during arousal from torpor [154]. Shivering is the most common method of producing heat in low temperatures but is not used in deep torpor because mechanisms that increase muscle function at low temperatures (e.g., a pool of inactive and available CK and HK) likely remain ineffective given the slowed rates of enzyme activity. A massive source of endogenous heat is needed to facilitate the shift in physiology and metabolic activity. Unlike the process of shivering, the energy released by oxidizing BAT is entirely heat [36]. Thus, the exclusively thermogenic result of oxidizing BAT is the key benefit of its use in arousal from hibernation.

Brown adipose tissue is under adrenergic control, and the β-adrenergic pathway in particular stimulates thermogenesis [36]. For reasons unknown, the signaling pathway that stimulates BAT thermogenesis in hibernators is more responsive as temperature drops. In Syrian hamsters (*Mesocricetus auratus*), stimulation of the β-adrenergic pathway at 36 °C produces no change in the metabolic activity of BAT in hibernating, nonhibernating, or cold-acclimated individuals, but stimulation at 12 °C results in an almost 200 % increase in hibernating animals and no change in the other groups [120]. Brown adipose tissue has a higher density of mitochondria than any other tissue and contains several smaller lipid droplets compared to a single large droplet as seen in WAT [36]. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is a co-regulator of metabolism that stimulates mitochondrial biogenesis and may play a key role in the number of mitochondria in bats. In little brown bats, PGC-1α is upregulated during hibernation to a higher degree in BAT than all other tissues [58]. In addition to occurring in higher density, mitochondria in BAT are specialized for heat production by the uncoupling of ATP production. Thermogenins (UCP-1) are found in high density in the mitochondria of BAT and allow electrons to bypass ATPase while re-entering the mitochondrial matrix [121]. The electron transport chain found in the mitochondria of BAT is also adapted for increased activity. For example, in hibernating little brown bats, expression of genes for some components of the electron transport chain in the membranes of BAT mitochondria is upregulated by as much as 400 % compared to other tissues during hibernation and all tissues in

euthermic conspecifics [57]. Thus, the capacity for oxidation and heat production is increased on the scale of each individual mitochondrion as well as BAT tissue in general. Taken together, BAT provides most hibernators with a mechanism for increased heat production heat at relatively low T_b compared to other tissues, including WAT and muscle.

A lack of BAT does not necessarily preclude the use of hibernation. Echidnas (*Tachyglossus aculeatus*; Order: Monotremata) and four species of pygmy possum (family Burramyidae; Infraorder: Marsupialia) are known to hibernate (reviewed in [84]); yet all lack functional BAT [94]. The patterns of arousals during hibernation in these Australasian species are similar to those of other hibernators and frequent arousals are observed [84], yet it appears they use WAT to fuel arousals, the mechanisms of which remain unknown. The small difference between active and hibernating MR and T_b in echidnas [156] might make it possible to rewarm using only WAT and shivering thermogenesis. In addition, the range of these hibernators is restricted to Australasia, and roosting or nesting ecology may partly explain persistence of arousals during hibernation despite lack of BAT, as is the case with the sole avian hibernator. The common poorwill roosts in areas exposed to sunlight and uses radiant solar heating to passively rewarm during arousals [206], which may negate a reliance on an endogenous heat source. Considerable energy can be saved by passively rewarming [80], and a minimum T_b threshold might be reached in doing so that would allow mechanisms for shivering thermogenesis to become functional, thus negating the reliance on BAT for rewarming. Common tenrecs (*Tenrec ecaudatus*), which are protoendothermic eutherians that have BAT, are known to go months in hibernation without arousing [137]. Given that T_a did not fall below 22 °C during this study, the lack of arousals suggests the physiological costs of hibernating (e.g., neurological degeneration or accumulation of metabolites) at such temperatures may not be substantial enough to require frequent arousals in this species. Interestingly, BAT found in the lesser hedgehog tenrec (*Echinops telfairi*) is not concentrated in the intrascapular region, as is typical in mammalian hibernators, but rather is found in the abdominal region and ostensibly used to maintain high T_b during reproduction [158]. The focused distribution of BAT in tenrecs may explain their inability to endogenously produce heat for arousal during hibernation. Although there are other adaptations that allow few hibernating animals to avoid the need for BAT, the predominant hibernation phenotype is characterized by long-term, deep, seasonal hibernation interspersed with frequent arousals, which are largely fueled by BAT.

Conclusion

Phenotypic plasticity is common, and hibernation is arguably one of the most profound examples among mammals. Adaptations associated with hibernation are multifaceted and allow for increased energy storage, pronounced metabolic depression and reduced T_b, selective maintenance of vital biological processes and cellular protection in an ischemic and hypothermic state, and the means for complete reversal to a euthermic state, all with virtually no pathological consequence. Hibernators become hyperphagic and considerably increase energy intake during the pre-hibernation period by way of seasonal resistance to hormones that typically inhibit appetite, dissociation of key hormones from providing adiposity feedback, and concomitant stimulation of lipid synthesis pathways. The switch in metabolic fuel during hibernation is facilitated by a decreased availability of carbohydrates and increased amounts of available lipids. Key enzymes that halt the flow of carbohydrates into metabolic pathways and stimulate the release of fatty acids for oxidation are upregulated during hibernation. Entrance into hibernation is influenced by photoperiod, temperature, and energy availability, and adaptations to the circadian system maintain semi-regular arousal patterns. However, the underlying intrinsic mechanism remains poorly understood.

The main purpose and consequence of hibernation is energy savings. Temperature-dependent effects on enzyme activity inherently decrease the rate, and therefore energy demand, of biological processes with falling T_b, but ATP turnover is further reduced physiologically. RPP plays a key role in activating and deactivating metabolic enzymes and substrates, such as PDH. Phosphorylation of enzymes involved in carbohydrate catabolism reduces the amount of substrate available for oxidation and consequently the rate of mitochondrial respiration and energy consumption. A large proportion of ATP turnover is also associated with maintenance of ion gradients across cellular membranes, and RPP of membrane proteins reduces activity and energy consumption, further facilitating metabolic reduction. The process of RPP is reversible given that it is a temporary modification and does not permanently change the substrate. Therefore, the pool of enzymes and proteins remains readily available and functional, even at euthermic temperatures. Although protein synthesis and biological processes are largely diminished and some even stopped during hibernation, tissue-specific changes maintain vital functions and protect tissues during this period of ischemia and hypothermia. For example, increased expression of SERCA2a in cardiac muscle facilitates cardiac function at lower temperatures [87], and mRNA is structurally changed to increase its stability during hibernation [122].

Membrane permeability is also maintained at a minimally functional level by shifts in the type of membrane fatty acids, an adaptation that avoids a counterproductive PUFA-associated increase in energy consumption. In addition, processes that normally occur during long periods of inactivity and lead to tissue damage are not evident during hibernation (e.g., lack of myostatin in skeletal muscle). Through a combination of protective adaptations and the ability to mute the body's normal, damaging responses, hibernators are able to mitigate the costs of low T_b, ischemia, and inactivity to realize the energetic benefits of quiescence and metabolic reduction.

The most intriguing aspect of hibernation is that adaptations for it involve modifications of existing physiological mechanisms that likely occur in all mammal lineages. Genes differentially expressed during hibernation that underlie the changes involved in hibernation physiology occur in all mammal lineages. Therefore, hibernation can be considered a seasonally expressed phenotype involving adaptive regulation of a number of pathways and processes that are evolutionarily conserved across all mammalian taxa. In fact, nonhibernators show similar responses to hypothermia and ischemia, as well as seasonal fluctuations in metabolism. WAT in some nonhibernators adapts to the cold by activating "brite" adipocytes, which express UCP-1 and increase thermogenic capacity [133]. Likewise, hypometabolism becomes evident and insulin resistance increases in some human populations in response to prolonged exposure to cold during winter [117]. However, hypothermic responses of nonhibernators are acute and typically differ from those of true hibernators, and are often followed by pathological consequences. Nonhibernators incur damage during periods of ischemia and hypothermia likely because they do not possess the entire suite of adaptations to cold and metabolic reduction as in hibernators. Physiological reductions to metabolism, maintenance of vital functions and protection of tissues in cold temperatures, and a means to warm up endogenously to resume function at normal temperatures are all necessary for the hibernation phenotype. However, some studies suggest an embryonic state of gene expression is achieved during hibernation. For example, moesin is only expressed in the enterocytes during fetal and hibernation states [38], and pancreatic lipase is found only in the pancreas of nonhibernators but throughout other tissues as well in fotuses of hibernators [7, 189]. Although heterothermy likely evolved separately in birds and mammals [77], there are examples of bird species using torpor early in development as altricial young but not in adulthood (e.g., [18]) and, with further study, we may find the same pattern in nonhibernating mammals. Perhaps many more mammals than we know are born with the ability to hibernate and to lose it with maturity. With a better understanding of the mechanisms involved in hibernation, at least some of the benefits seen during torpor, such as resistance to metabolic disease despite adiposity (e.g., [155]), may be achievable in nonhibernators (e.g., humans), a prospect that adds urgency and excitement to further studies in hibernation physiology.

Glossary

Daily torpor	Short-term employment of torpor characterized by bouts typically lasting less than 24 h with body temperature (T_b) often remaining some degrees above ambient temperature (T_a).
Endothermy	Regulation of T_b using a high rate of metabolism to produce endogenous heat.
Euthermy	Maintenance of T_b at a relatively high temperature conducive to normal biological function.
Heterothermy	The diel or seasonal pattern of temperature regulation where T_b varies outside the normal euthermic range
Hibernation	Extended employment of torpor characterized by bouts typically lasting days to weeks with T_b dropping to near T_a. Obligate hibernation lasts for months during predictable periods of energy imbalance (the "hibernation season") and consists of multiple bouts interspersed with regular arousals. Conversely, facultative hibernation typically occurs during ephemeral energy crises outside of any predictable season
Hypothermia	An uncontrolled state of reduced T_b (i.e., heat loss exceeds capacity for heat production)
Torpor	A temporary and controlled reduction in metabolic rate (MR) and body temperature (T_b) followed by a return to euthermy using an endogenous source of heat. Torpor is often employed as an adaptive response to conserve energy during periods of resource scarcity

References

1. Abnous K, Storey KB (2007) Regulation of skeletal muscle creatine kinase from a hibernating mammal. Arch Biochem Biophys 467:10–19
2. Abnous K, Storey KB (2008) Skeletal muscle hexokinase: regulation in mammalian hibernation. Mol Cell Biol 319:41–50
3. Allan SM, Rothwell NJ (2003) Inflammation in central nervous system injury. Philos Trans R Soc Lond B 358:1669–1677
4. Aloia RC, Raison JK (1989) Membrane function in mammalian hibernation. Biochemica et Biophysica Acta 988:123–146

5. Andrews MT (2004) Genes controlling the metabolic switch in hibernating mammals. Biochem Soc Trans 32:1021–1024
6. Andrews MT (2007) Advances in the molecular biology of hibernation in mammals. BioEssays 29:431–440
7. Andrews MT, Squire TL, Bowen CM, Rollins MB (1998) Low-temperature carbon utilization is regulated by novel gene activity in the heart of a hibernating mammal. Proc Natl Acad Sci 95:8392–8397
8. Angilletta MJ, Cooper BS, Schuler MS, Boyles JG (2010) The evolution of thermal physiology in endotherms. Front Biosci E2:861–881
9. Armitage KB, Blumstein DT, Woods BC (2003) Energetics of hibernating yellow-bellied marmots (*Marmota flaviventris*). Comp Biochem Physiol 134A:101–114
10. Baker CJ, Flore AJ, Frazzini VI, Choudhri TF, Zubay GP, Solomon RA (1995) Intraischemic hypothermia decreases the release of glutamate in the cores of permanent focal cerebral infarcts. Neurosurgery 36:994–1001
11. Barnes BM (1989) Freeze avoidance in a mammal: body temperatures below 0°C in an Arctic hibernator. Science 244:1593–1595
12. Barnes BM, Kretzmann M, Licht P, Zucker I (1986) The influence of hibernation on testis growth and spermatogenesis in the golden-mantled ground squirrel, *Spermophilus lateralis*. Biol Reprod 35:1289–1297
13. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism and function. Cell 116:281–297
14. Bauman WA (1990) Seasonal changes in pancreatic insulin and glucagon in the little brown bat (*Myotis lucifugus*). Pancreas 5:342–346
15. Bintz GL, Mackin WW (1980) The effect of water availability on tissue catabolism during starvation in Richardson's ground squirrels. Comp Biochem Physiol 65A:181–186
16. Bintz GL, Palmer DL, Mackin WW, Blanton FY (1979) Selective tissue catabolism and water balance during starvation in Richardson's ground squirrels. Comp Biochem Physiol 64A:399–403
17. Blake BH (1972) The annual cycle and fat storage in two populations of golden-mantled grounds squirrels. J Mammal 53:157–167
18. Boersma PD (1986) Body temperature, torpor, and growth in chicks of fork-tailed strom-petrels (*Oceanodroma furcata*). Physiol Zool 59:10–19
19. Boonstra R, Mo K, Monks DA (2014) Managing anabolic steroids in pre-hibernating Arctic ground squirrels: obtaining their benefits and avoiding their costs. Biol Lett 10:20140734
20. Born J, Rasch B, Gais S (2006) Sleep to remember. Neuroscientist 12:410–424
21. Boswell T, Woods SC, Kenagy GJ (1994) Seasonal changes in body mass, insulin, and glucocorticoids of free-living golden-mantled ground squirrels. Gen Comp Endocrinol 96:339–346
22. Bouma HR, Carey HV, Kroese FGM (2010) Hibernation: the immune system at rest? J Leukoc Biol 88:619–624
23. Bouma HR, Strijkstra AM, Talaei F, Henning RH, Carey HV, Kroese FGM (2012) The hibernating immune system. In: Ruf T, Bieber C, Arnold W, Millesi E (eds) Living in a seasonal world: thermoregulatory and metabolic adaptations. Springer-Verlag, Berlin, pp 259–270
24. Bramlett HM, Dietrich WD (2004) Pathophysiology of cerebral ischemia and brain trauma: similarities and differences. J Cereb Blood Flow Metab 24:133–150
25. Brauch KM, Dhruv ND, Hanse EA, Andrews MT (2005) Digital transcriptome analysis indicates adaptive mechanisms in the heart of a hibernating mammal. Physiol Genomics 23:227–234
26. Brigham RM, Ianuzzo CD, Hamilton N, Fenton MB (1990) Histochemical and biochemical plasticity of muscle fibers in the little brown bat (*Myotis Iucifugus*). J Comp Physiol B 160:183–186
27. Brigham RM, McKechnie AE, Doucette LI, Geiser F (2012) Heterothermy in caprimulgid birds: a review of inter- and intraspecific variation in free-ranging populations. In: Ruf T, Bieber C, Arnold W, Millesi E (eds) Living in a seasonal world: thermoregulatory and metabolic adaptations. Springer-Verlag, Berlin, pp 175–187
28. Brooks NE, Myburgh KH, Storey KB (2011) Myostatin levels in skeletal muscle of hibernating ground squirrels. J Exp Biol 214:2522–2527
29. Brooks SPJ, Storey KB (1992) Mechanisms of glycolytic control during hibernation in the ground squirrel *Spermophilus lateralis*. J Comp Physiol B 162:23–28
30. Brunet-Rossinni AK (2004) Reduced free-radical production and extreme longevity in the little brown bat (*Myotis lucifugus*) versus two non-flying mammals. Mech Ageing Dev 125:11–20
31. Buck CL, Barnes BM (1999) Annual cycle of body composition and hibernation in free-living Arctic ground squirrels. J Mammal 80:430–442
32. Buck CL, Barnes BM (2000) Effects of ambient temperature on metabolic rate, respiratory quotient, and torpor in an Arctic hibernator. Am J Physiol Regul Integr Comp Physiol 279:R255–R262
33. Burlington RF, Darvish A (1988) Low-temperature performance of isolated working hearts from a hibernator and a nonhibernator. Physiol Zool 61:387–395
34. Burton RS, Reichman OJ (1999) Does immune challenge affect torpor duration? Funct Ecol 13:232–237
35. Cammisotto PG, Bendayan M (2007) Leptin secretion by white adipose tissue and gastric mucosa. Histol Histopathol 22:199–210
36. Cannon B, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. Physiol Rev 84:277–359
37. Carey HV (1990) Seasonal changes in mucosal structure and function in ground squirrel intestine. Am J Physiol Regul Integr Comp Physiol 259:R385–R392
38. Carey HV, Mangino MJ, Southard JH (2001) Changes in gut function during hibernation: implications for bowel transplantation and surgery. Gut 49:459–461
39. Carey HV, Martin SL (1996) Preservation of intestinal gene expression during hibernation. Am J Physiol Gastrointest Liver Physiol 271:G804–G813
40. Carey HV, Sills NS (1992) Maintenance of intestinal nutrient transport during hibernation. Am J Physiol Regul Integr Comp Physiol 263:R517–R523
41. Chan JA, Krichevsky AM, Kosik KS (2005) MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 65:6029–6033
42. Chapman D (1975) Phase transitions and fluidity characteristics of lipids and cell membranes. Q Rev Biophys 8:185–235
43. Chen JF et al (2006) The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nat Genet 38:228–233
44. Clemens LE, Heldmaier G (2009) Keep cool: memory is retained during hibernation in Alpine marmots. Physiol Behav 98:78–84
45. Concannon P, Levac K, Rawson R, Tennant B, Bensadoun A (2001) Seasonal changes in serum leptin, food intake, and body weight in photoentrained woodchucks. Am J Physiol Regul Integr Comp Physiol 281:R951–R959
46. Cossins AR, Prosser CL (1978) Evolutionary adaptation of membranes to temperature. Proc Natl Acad Sci 75:2040–2043
47. Cotton CJ, Harlow HJ (2010) Avoidance of skeletal muscle atrophy in spontaneous and facultative hibernators. Physiol Biochem Zool 83:551–560

48. Cummings DE (2006) Ghrelin and the short- and long-term regulation of appetite and body weight. Physiol Behav 89:71–84
49. Currie SE, Körtner G, Geiser F (2014) Heart rate as a predictor of metabolic rate in heterothermic bats. J Exp Biol 217:1519–1524
50. Currie SE, Noy K, Geiser F (2015) Passive rewarming from torpor in hibernating bats: minimizing metabolic costs and cardiac demands. Am J Physiol Regul Integr Comp Physiol 308:R34–R41
51. Czenze ZJ, Park AD, Willis CKR (2013) Staying cold through dinner: cold-climate bats rewarm with conspecifics but not sunset during hibernation. J Comp Physiol B 183:859–866
52. Dausmann KH (2014) Flexible patterns in energy savings: heterothermy in primates. J Zool 292:101–111
53. Davis WH, Reite OB (1967) Response of bats from temperate regions to changes in ambient temperature. Biol Bull 132:320–328
54. de Meis L (2002) Ca^{2+}-ATPases (SERCA): energy transduction and heat production in transport. J Membr Biol 188:1–9
55. Deboer T, Tobler I (1994) Sleep EEG after daily torpor in the Djungarian hamster: similarity to the effects of sleep deprivation. Neurosci Lett 166:35–38
56. Dunbar MB, Tomasi TE (2006) Arousal patterns, metabolic rate, and an energy budget of eastern red bats (*Lasiurus borealis*) in winter. J Mammal 87:1096–1102
57. Eddy SF, Morin PJ, Storey KB (2006) Differential expression of selected mitochondrial genes in hibernating little brown bats, *Myotis lucifugus*. J Exp Zool 305A:620–630
58. Eddy SF, Storey KB (2003) Differential expression of Akt, PPARγ, and PGC-1 during hibernation in bats. Biochem Cell Biol 81:269–274
59. Eddy SF, Storey KB (2007) $p38^{MAPK}$ regulation of transcription factor targets in muscle and heart of the hibernating bat, *Myotis lucifugus*. Cell Biochem Funct 25:759–765
60. Ellory JC, Willis JS (1982) Kinetics of the sodium pump in red cells of different temperature sensitivity. J Gen Physiol 79:1115–1130
61. Else PL, Turner N, Hulbert AJ (2004) The evolution of endothermy: role for membranes and molecular activity. Physiol Biochem Zool 77:950–958
62. Epperson LE, Martin SL (2002) Quantitative assessment of ground squirrel mRNA levels in multiple stages of hibernation. Physiol Genomics 10:93–102
63. Epperson LE, Rose JC, Carey HV, Martin SL (2010) Seasonal proteomic changes reveal molecular adaptations to preserve and replenish liver proteins during ground squirrel hibernation. Am J Physiol Regul Integr Comp Physiol 298:R329–R340
64. Esau C et al (2006) miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab 3:87–98
65. Florant GL, Healy JE (2012) The regulation of food intake in mammalian hibernators: a review. J Comp Physiol B 182:451–467
66. Florant GL, Lawrence AK, Williams K, Bauman WA (1985) Seasonal changes in pancreatic B-cell function in euthermic yellow-bellied marmots. Am J Physiol Regul Integr Comp Physiol 249:R159–R165
67. Florant GL et al (2004) Fat-cell mass, serum leptin and adiponectin changes during weight gain and loss in yellow-bellied marmots (*Marmota flaviventris*). J Comp Physiol B 174:633–639
68. Florant GL, Richter MM, Fried SK (2012) The effect of ambient temperature on body mass, torpor, food intake, and leptin levels: implications on the regulation of food intake in mammalian hibernators. In: Ruf T, Bieber C, Arnold W, Millesi E (eds) Living in a seasonal world: thermoregulatory and metabolic adaptations. Springer-Verlag, Berlin, pp 507–517
69. Frank CJ (1992) The influence of dietary fatty acids on hibernation by golden-mantled ground squirrels (*Spermophilus lateralis*). Physiol Zool 65:906–920
70. Frank CL, Storey KB (1995) The optimal depot fat composition for hibernation by golden-mantled ground squirrels (*Spermophilus lateralis*). J Comp Physiol B 164:536–542
71. French AR (1988) The patterns of mammalian hibernation. Am Sci 76:569–575
72. French AR (2008) Patterns of heterothermy in rodents. In: Lovegrove BG, McKechnie AE (eds) Hypometabolism in animals: torpor, hibernation and cryobiology. 13th International Hibernation Symposium. University of KwaZulu-Natal, Pietermaritzburg, pp 337–352
73. Frerichs KU et al (1998) Suppression of protein synthesis in brain during hibernation involves inhibition of protein initiation and elongation. Proc Natl Acad Sci 95:14511–14516
74. Geiser F (1988) Reduction of metabolism during hibernation and daily torpor in mammals and birds: temperature effect or physiological inhibition? J Comp Physiol B 158:25–37
75. Geiser F (1991) The effect of unsaturated and saturated dietary lipids on the pattern of daily torpor and the fatty acid composition of tissues and membranes of the deer mouse *Peromyscus maniculatus*. J Comp Physiol B 161:590–597
76. Geiser F (2004) Metabolic rate and body temperature reduction during hibernation and daily torpor. Annu Rev Physiol 66:239–274
77. Geiser F (2008) Ontogeny and phylogeny of endothermy and torpor in mammals and birds. Comp Biochem Physiol 150A:176–180
78. Geiser F, Broome LS (1993) The effect of temperature on the pattern of torpor in a marsupial hibernator. J Comp Physiol B 163:133–137
79. Geiser F, Currie SE, O'Shea KA, Hiebert SM (2014) Torpor and hyporthemia: reversed hysteresis of metabolic rate and body temperature. Am J Physiol Regul Integr Comp Physiol 307:R1324–R1329
80. Geiser F, Drury RL (2003) Radiant heat affects thermoregulation and energy expenditure during rewarming from torpor. J Comp Physiol B 173:55–60
81. Geiser F, Kenagy GJ (1987) Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator. Am J Physiol Regul Integr Comp Physiol 252:R897–R901
82. Geiser F, Kenagy GJ (1988) Torpor duration in relation to temperature and metabolism in hibernating ground squirrels. Physiol Zool 61:422–449
83. Geiser F, Kenagy GJ, Wingfield JC (1997) Dietary cholesterol enhances torpor in a rodent hibernator. J Comp Physiol B 167:416–422
84. Geiser F, Körtner G (2010) Hibernation and daily torpor in Australian mammals. Aust Zool 35:204–215
85. Geiser F, Ruf T (1995) Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns. Physiol Zool 68:935–966
86. Girdwood DW, Tatham MH, Hay RT (2004) SUMO and transcriptional regulation. Semin Cell Dev Biol 15:201–210
87. Giroud S, Frare C, Strijkstra A, Boerema A, Arnold W, Ruf T (2013) Membrane phospholipid fatty acid composition regulates cardiac SERCA activity in a hibernator, the Syrian hamster (*Mesocricetus auratus*). PLoS ONE 8:e63111
88. Hall AC, Wolowyk MW, Wang LCH, Ellory JC (1987) The effects of temperature on Ca^{2+} transport in red cells from a hibernator (*Spermophilus richardsonii*). J Therm Biol 12:61–63
89. Halsall AL, Boyles JG, Whitaker JO Jr (2012) Body temperature of big brown bats during winter in a building hibernaculum. J Mammal 93:497–503

90. Hardie DG, Hawley SA, Scott JW (2006) AMP-activated protein kinase—development of the energy sensor concept. J Physiol 574:7–15
91. Hardy RN (1979) Temperature and animal life, 2nd edn. Edward Arnold, London
92. Hayashi K, Kugimiya M, Funatsu M (1968) Heat stability of lysozyme-substrate complex. J Biochem 64:93–97
93. Hays GC, Webb PI, Speakman JR (1991) Arrhythmic breathing in torpid pipistrelle bats, *Pipistrellus pipistrellus*. Respir Physiol 85:185–192
94. Hayward JS, Lisson PA (1992) Evolution of brown fat: its absence in marsupials and monotremes. Can J Zool 70:171–179
95. Hazel JR (1995) Thermal adaptations in biological membranes: is homeoviscous adaptation the explanation? Annu Rev Physiol 57:19–42
96. Healy JE, Florant GL (2012) Ghrelin, leptin, and fatty acids in free-living *Callospermophilus lateralis* (golden-mantled ground squirrels). In: Ruf T, Bieber C, Arnold W, Millesi E (eds) Living in a seasonal world: thermoregulatory and metabolic adaptations. Springer-Verlag, Berlin, pp 519–529
97. Healy JE, Bateman JL, Ostrom CE, Florant GL (2011) Peripheral ghrelin stimulates feeding behavior and positive energy balance in a sciurid hibernator. Horm Behav 59:512–519
98. Healy JE, Gearhart CN, Bateman JL, Handa RJ, Florant GL (2011) AMPK and ACC change with fasting and physiological condition in euthermic and hibernating golden-mantled ground squirrels (*Callospermophilus lateralis*). Comp Biochem Physiol A 159:322–331
99. Healy JE, Ostrom CE, Wilkerson GK, Florant GL (2010) Plasma ghrelin concentrations change with physiological state in a sciurid hibernator (*Spermophilus lateralis*). Gen Comp Endocrinol 166:372–378
100. Heldmaier G, Elvert R (2004) How to enter torpor: thermodynamic and physiological mechanisms of metabolic depression. In: Barnes BM, Carey HV (eds) Life in the cold: evolution, mechanisms, adaptation, and application. Twelfth International Hibernation Symposium. Institute of Arctic Biology, University of Alaska, Fairbanks, pp 185–198
101. Heller HC (1979) Hibernation: neural aspects. Annu Rev Physiol 41:305–321
102. Heller HC, Colliver GW, Beard J (1977) Thermoregulation during entrance into hibernation. Pflügers Archiv 369:55–59
103. Hermanson JW (2009) Cool muscle properties in hibernating and summer *Eptesicus fuscus* in New York. Bat Res News 50:111
104. Hillenius WJ, Ruben JA (2004) The evolution of endothermy in terrestrial vertebrates: Who? When? Why? Physiol Biochem Zool 77:1019–1042
105. Hindle AG, Otis JP, Epperson LE, Hornberger TA, Goodman CA, Carey HV, Martin SL (2014) Prioritization of skeletal muscle growth for emergence from hibernation. J Exp Biol. doi:10.1242/jeb.109512
106. Hittle D, Storey KB (2002) The translation state of differentially expressed mRNAs in the hibernating thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*). Arch Biochem Biophys 401:244–254
107. Hope PR, Jones G (2013) An entrained circadian cycle of peak activity in a population of hibernating bats. J Mammal 94:497–505
108. Horman S, Hussain N, Dilworth SM, Storey KB, Rider MH (2005) Evaluation of the role of AMP-activated protein kinase and its downstream targets in mammalian hibernation. Comp Biochem Physiol B 142:374–382
109. Hulbert AJ (2007) Membrane fatty acids as pacemakers of animal metabolism. Lipids 42:811–819
110. Hulbert AJ, Else PL (2000) Mechanisms underlying the cost of living in animals. Annu Rev Physiol 62:207–235
111. Hume ID, Beiglböck C, Ruf T, Frey-Roos F, Bruns U, Arnold W (2002) Seasonal changes in morphology and function of the gastrointestinal tract of free-living alpine marmots (*Marmota marmota*). J Comp Physiol B 172:197–207
112. Humphries MM, Thomas DW, Kramer DL (2003) The role of energy availability in mammalian hibernation: a cost-benefit approach. Physiol Biochem Zool 76:165–179
113. Iliff BW, Swoap SJ (2012) Central adenosine receptor signaling is necessary for daily torpor in mice. Am J Physiol Regul Integr Comp Physiol 303:R477–R484
114. IUPS Thermal Commission (2003) Glossary of terms for thermal physiology. J Therm Biol 28:75–106
115. Jackman RW, Kandarian SC (2004) The molecular basis of skeletal muscle atrophy. Am J Physiol Cell Physiol 287:834–843
116. Jinka TR, Tøien Ø, Drew KL (2011) Season primes the brain in an Arctic hibernator to facilitate entrance into torpor mediated by adenosine A_1 receptors. J Neurosci 31:10752–10758
117. Kamezaki F et al (2013) Association of seasonal variation in the prevalence of metabolic syndrome with insulin resistance. Hypertens Res 36:398–402
118. Kelly DA, Storey KB (1995) Glycolysis and energetics in organs of hibernating mice (*Zapus hudsonius*). Can J Zool 73:202–207
119. Kenagy GJ (1980) Effects of day length, temperature, and endogenous control on annual rhythms of reproduction and hibernation in chipmunks (*Eutamias spp.*). J Comp Physiol A 141:369–378
120. Kitao N, Hashimoto M (2012) Increased thermogenic capacity of brown adipose tissue under low temperature and its contribution to arousal from hibernation in Syrian hamsters. Am J Physiol Regul Integ Comp Physiol 302:R118–R125
121. Klaus S, Casteilla L, Bouillaud F, Ricquier D (1991) The uncoupling protein UCP: a membraneous mitochondrial ion carrier exclusively expressed in brown adipose tissue. Int J Biochem 23:791–801
122. Knight JE, Narus EN, Martin SL, Jacobson A, Barnes BM, Boyer BB (2000) mRNA stability and polysome loss in hibernating Arctic ground squirrels (*Spermophilus parryii*). Mol Cell Biol 20:6374–6379
123. Kola B (2008) Role of AMP-activated Protein Kinase in the control of appetite. J Neuroendocrinol 20:942–951
124. Körtner G, Geiser F (2000) The temporal organization of daily torpor and hibernation: circadian and circannual rhythms. Chronobiol Int 17:103–128
125. Krilowicz BL, Glotzbach SF, Heller HC (1988) Neuronal activity during sleep and complete bouts of hibernation. Am J Physiol Regul Integr Comp Physiol 255:R1008–R1019
126. Kronfeld-Schor N, Richardson C, Silvia BA, Kunz TH, Widmaier EP (2000) Dissociation of leptin secretion and adiposity during prehibernatory fattening in little brown bats. Am J Physiol Regul Integr Comp Physiol 279:R1277–R1281
127. Krüger K, Prinzinger R, Schuchmann K-L (1982) Torpor and metabolism in hummingbirds. Comp Biochem Physiol 73A:679–689
128. Kunz TH, Wrazen JA, Burnett CD (1998) Changes in body mass and fat reserves in pre-hibernating little brown bats (*Myotis lucifugus*). Ecoscience 5:8–17
129. Lal A et al (2009) miR-24 inhibits cell proliferation by targeting E2F2, MYC, and other cell-cycle genes via binding to "Seedless" 3'UTR microRNA recognition elements. Mol Cell 35:610–625
130. Lee K, Park JY, Yoo W, Gwag T, Lee J-W, Byun M-W, Choi I-H (2008) Overcoming muscle atrophy in a hibernating

mammal despite prolonged disuse in dormancy; proteomic and molecular assessment. J Cell Biochem 104:642–656
131. Lee Y, Miyake S, Wakita H, McMullen DC, Azuma Y, Auh S, Hallenbeck JM (2007) Protein SUMOylation is massively increased in hibernation torpor and is critical for the cytoprotection provided by ischemic preconditioning and hypothermia in SHSY5Y cells. J Cereb Blood Flow Metab 27:950–962
132. Letunic I, Bork P (2007) Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23:127–128
133. Li Y, Lasar D, Fromme T, Klingenspor M (2014) White, brite, and brown adipocytes: the evolution and function of a heater organ in mammals. Can J Zool 92:615–626
134. Liu B, Belke D, Wang LCH (1997) Ca^{2+} uptake by cardiac sarcoplasmic reticulum at low temperature in rat and ground squirrel. Am J Physiol Regul Integr Comp Physiol 272:R1121–R1127
135. Lotter H, Helk E, Bernin H, Jacobs T, Prehn C, Adamski J, González-Roldán N, Holst O, Tannich E (2013) Testosterone increases susceptibility to amebic liver abscess in mice and mediates inhibition of IFNγ secretion in natural killer T cells. PLoS ONE 8:e55694
136. Lovegrove BG (2012) A single origin of heterothermy in mammals. In: Ruf T, Bieber C, Arnold W, Millesi E (eds) Living in a seasonal world; thermoregulatory and metabolic adaptations. Springer-Verlag, Berlin, pp 3–11
137. Lovegrove BG, Lobban KD, Levesque DL (2014) Mammal survival at the Cretaceous-Palaeogene boundary: metabolic homeostasis in prolonged tropical hibernation in tenrecs. Proc R Soc B 281:20141304
138. Lusk G (1924) Animal calorimetry: analysis of the oxidation of mixtures of carbohydrate and fat. J Biol Chem 59:41–42
139. Lyman CP (1982) The hibernating state. In: Lyman P, Willis JS, Malan A, Wang LCH (eds) Hibernation and torpor in mammals and birds. Academic Press, New York, pp 54–76
140. Magariños AM, McEwan BS, Saboureau M, Pevet P (2006) Rapid and reversible changes in intrahippocampal connectivity during the course of hibernation in European hamsters. Proc Natl Acad Sci 103:18775–18780
141. Malan A (2012) The torpor-arousal cycle is controlled by an endogenous clock. In: Ruf T, Bieber C, Arnold W, Millesi E (eds) Living in a seasonal world: thermoregulatory and metabolic adaptations. Springer-Verlag, Berlin, pp 211–218
142. Malysheva AN, Storey KB, Ziganshin RK, Lopina OD, Rubtsov AM (2001) Characteristics of sarcoplasmic reticulum membrane preparations isolated from skeletal muscles of active and hibernating ground squirrel *Spermophilus undulatus*. Biochemistry 66:918–925
143. Marjanovic M, Willis JS (1992) ATP dependence of Na^{+}-K^{+} pump of cold-sensitive and cold-tolerant mammalian red blood cells. J Physiol 456:575–590
144. McNamara MC, Riedesel ML (1973) Memory and hibernation in *Citellus lateralis*. Science 179:92–94
145. Mihailovic L, Petrovoc-Minic B, Protic S, Divac J (1968) Effects of hibernation on learning and retention. Nature 218:191–192
146. Millesi E, Prossinger H, Dittami JP, Fieder M (2001) Hibernation effects on memory in European ground squirrels (*Spermophilus citellus*). J Biol Rhythms 16:264–271
147. Minokoshi Y, Shiuchi T, Lee S, Suzuki A, Okamoto S (2008) Role of hypothalamic AMP-kinase in food regulation. Nutrition 24:786–790
148. Morin PJ, Dubuc A, Storey KB (2008) Differential expression of microRNA species in organs of hibernating ground squirrels: a role in translational suppression during torpor. Biochemica et Biophysica Acta 1779:628–633
149. Morrison SF, Nakamura K (2011) Central neural pathways for thermoregulation. Front Biosci 16:74–104
150. Morton SR (1978) Torpor and nest-sharing in free-living *Sminthopsis crassicaudata* (Marsupialia) and *Mus musculus* (Rodentia). J Mammal 59:569–575
151. Munro D, Thomas DW (2004) The role of polyunsaturated fatty acids in the expression of torpor by mammals: a review. Zoology 107:29–48
152. Musacchia XJ, Steffen JM, Fell RD, Dombrowski MJ (1990) Skeletal muscle response to spaceflight, whole body suspension, and recovery in rats. J Appl Physiol 69:2248–2253
153. Muzzi M, Blasi F, Masi A, Coppi E, Traini C, Felici R, Pittelli M, Cavone L, Pugliese AM, Moroni F, Chiarugi A (2013) Neurological basis of AMP-dependent thermoregulation and its relevance to central and peripheral hyperthermia. J Cereb Blood Flow Metab 33:183–190
154. Nedergaard J, Cannon B (1984) Preferential utilization of brown adipose tissue lipids during arousal from hibernation in hamsters. Am J Physiol Regul Integr Comp Physiol 247:R506–R512
155. Nelson OL, Jansen HT, Galbreath E, Morgenstern K, Gehring JL, Rigano KS, Lee J, Gong J, Shaywitz AJ, Vella CA, Robbins CT, Corbit KC (2014) Grizzly bears exhibit augmented insulin sensitivity while obese prior to a reversible insulin resistance during hibernation. Cell Metab 20:376–382
156. Nicol SC, Andersen NA, Mesch U (1992) Metabolic rate and ventilatory pattern in the echidna during hibernation and arousal. In: Augee ML (ed) Platypus and Echidnas. Royal Zoological Society of New South Wales, Sydney, pp 150–159
157. Norquay KJO, Willis CKR (2014) Hibernation phenology of *Myotis lucifugus*. J Zool. doi:10.1111/jzo.12155
158. Oelkrug R, Goetze N, Exner C, Lee Y, Ganjam GK, Kutschke M, Müller S, Stöhr S, Tschöp MH, Crichton PG, Heldmaier G, Jastroch M, Meyer CW (2013) Brown fat in a protoendothermic mammal fuels eutherian evolution. Nat Commun 4:1–8
159. Offner H, Subramanian S, Parker SM, Afentoulis ME, Vandenbark AA, Hurn PD (2006) Experimental stroke induces massive, rapid activation of the peripheral immune system. J Cereb Blood Flow Metab 26:654–665
160. Ormseth OA, Nicolson M, Pelleymounter M, Boyer BB (1996) Leptin inhibits prehibernation hyperphagia and reduces body weight in Arctic ground squirrels. Am J Physiol Regul Integr Comp Physiol 40:R1775–R1779
161. Otis JP, Sahoo D, Drover VA, Yen CE, Carey HV (2011) Cholesterol and lipoprotein dynamics in a hibernating mammal. PLoS ONE 6:e29111
162. Pengelley ET, Asmundson SJ, Barnes B, Aloia RC (1976) Relationship of light intensity and photoperiod to circannual rhythmicity in the hibernating ground squirrel, *Citellus lateralis*. Comp Biochem Physiol A 53:273–277
163. Popov VI, Bocharova LS, Bragin AG (1992) Repeated changes of dendritic morphology in the hippocampus of ground squirrels in the course of hibernation. Neuroscience 48:45–51
164. Prendergast BJ, Freeman DA, Zucker I, Nelson RJ (2002) Periodic arousal from hibernation is necessary for initiation of immune responses in ground squirrels. Am J Physiol Regul Integr Comp Physiol 282:R1054–R1062
165. Revel FG et al (2007) The circadian clock stops ticking during deep hibernation in the European hamster. Proc Natl Acad Sci 104:13816–13820
166. Reznick RM, Shulman GI (2006) The role of AMP-activated protein kinase in mitochondrial biogenesis. J Physiol 574:33–39
167. Riek A, Geiser F (2014) Heterothermy in pouched mammals—a review. J Zool 292:74–85
168. Riley DA, Ilyina-Kakueva EI, Ellis S, Bain JLW, Slocum GR, Sedlak FR (1990) Skeletal muscle fiber, nerve, and blood vessel breakdown in space-flown rats. FASEB J 4:84–91

169. Rolfe DFS, Brown GC (1997) Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol Rev 77:731–758
170. Ross AP, Christian SL, Zhao HW, Drew KL (2006) Persistent tolerance to oxygen and nutrient deprivation and N-methyl-D-aspartate in cultured hippocampal slices from hibernating Arctic ground squirrel. J Cereb Blood Flow Metab 26:1148–1156
171. Rousseau K et al (2002) Photoperiodic regulation of leptin resistance in the seasonally breeding Siberian hamster (*Phodopus sungorus*). Endocrinology 143:3083–3095
172. Rousseau K, Atcha Z, Loudon ASI (2003) Leptin and seasonal mammals. J Neuroendocrinol 15:409–414
173. Ruby NF (2003) Hibernation: when good clocks go cold. J Biol Rhythms 18:275–286
174. Ruczynski I, Siemers BM (2014) Hibernation does not affect memory retention in bats. Biol Lett 7:153–155
175. Ruf T, Arnold W (2008) Effects of polyunsaturated fatty acids on hibernation and torpor: a review and hypothesis. Am J Physiol Regul Integr Comp Physiol 294:R1044–R1052
176. Ruf T, Geiser F (2014) Daily torpor and hibernation in birds and mammals. Biol Rev. doi:10.1111/brv.12137
177. Sheriff MJ, Fridinger RW, Tøien Ø, Barnes BM, Buck CL (2013) Metabolic rate and prehibernation fattening in free-living Arctic ground squirrels. Physiol Biochem Zool 86:515–527
178. Sinensky M (1974) Homeoviscous adaptation—a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. Proc Natl Acad Sci 71:522–525
179. Singer SJ, Nicolson GL (1972) The fluid mosaic model of the structure of cell membranes. Science 175:720–731
180. Srere HK, Belke D, Wang LCH, Martin SL (1995) α2-macroglobulin gene expression is independent of acute phase response during hibernation in ground squirrels. Am J Physiol Regul Integr Comp Physiol 268:R1507–R1512
181. Srere HK, Wang LCH, Martin SL (1992) Central role for differential gene expression in mammalian hibernation. Proc Natl Acad Sci 89:7119–7123
182. Stawski C, Willis CKR, Geiser F (2014) The importance of temporal heterothermy in bats. J Zool 292:86–100
183. Stephens TW, Caro JF (1998) To be lean or not to be lean: is leptin the answer? Exp Clin Endocrinol Diabetes 106:1–15
184. Storey KB (2010) Out cold: biochemical regulation of mammalian hiberntion—a mini-review. Gerontology 56:220–230
185. Storey KB, Storey JM (2004) Metabolic rate depression in animals: transcriptional and translational controls. Biol Rev Camb Philos Soc 79:207–233
186. Strijkstra A, Koopmans T, Bouma HR, de Boer SF, Hut RA, Boerema AS (2012) On the dissimilarity of 5'-AMP induced hypothermia and torpor in mice. In: Ruf T, Bieber C, Arnold W, Millesi E (eds) Living in a seasonal world: thermoregulatory and metabolic adaptations. Springer-Verlag, Berlin, pp 351–362
187. Swoap SJ, Rathvon M, Gutilla M (2007) AMP does not induce torpor. Am J Physiol Regul Integr Comp Physiol 293:R468–R473
188. Tähti H (1978) Seasonal differences in O_2 consumption and respiratory quotient in a hibernator (*Erinaceus europaeus* L.). Ann Zool Fenn 15:69–75
189. Terada T, Nakanuma Y (1995) Expression of pancreatic enzymes (a-amylase, trypsinogen, and lipase) during human liver development and maturation. Gastroenterology 108:1236–1245
190. Thomas DW (1995) Hibernating bats are sensitive to nontactile human disturbance. J Mammal 76:940–946
191. Tomanek RJ, Lund DD (1974) Degeneration of different types of skeletal muscle fibres; II. Immobilization. J Anat 118:531–541
192. Tschöp M, Smiley DL, Heiman ML (2000) Ghrelin induces adiposity in rodents. Nature 407:908–913
193. Tupone D, Madden CJ, Morrison SF (2013) Central activation of the A1 adenosine receptor (A1AR) induces a hypothermic, torpor-like state in the rat. J Neurosci 33:14512–14525
194. Tyml K, Mathieu-Costello O (2001) Structural and functional changes in the microvasculature of disused skeletal muscle. Front Biosci 6:D45–D52
195. van Breukelen F, Martin SL (2002) Reversible depression of transcription during hibernation. J Comp Physiol B 172:355–361
196. Walker JM, Glotzbach SF, Berger RJ, Heller HC (1977) Sleep and hibernation in ground squirrels (*Citellus spp*): electrophysiological observations. Am J Physiol Regul Integr Comp Physiol 233:R213–R221
197. Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM (1992) Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the "phosphocreatine circuit" for cellular energy homeostasis. Biochem J 281:21–40
198. Wang LCH, Wolowyk M (1988) Torpor in mammals and birds. Can J Zool 66:133–137
199. Wang LCH, Cao HM, Zhou ZQ (1997) Temperature dependence of the myocardial excitability of ground squirrel and rat. J Therm Biol 22:195–199
200. Wang LCH, Lakatta EG, Cheng H, Zhou ZQ (2002) Adaptive mechanisms of intracellular calcium homeostasis in mammalian hibernators. J Exp Biol 205:2957–2962
201. Williams CT, Barnes BM, Kenagy GJ, Buck CL (2014) Phenology of hibernation and reproduction in ground squirrels: integration of environmental cues with endogenous programming. J Zool 292:112–124
202. Willis CKR, Brigham RM, Geiser F (2006) Deep, prolonged torpor by pregnant, free-ranging bats. Naturwissenschaften 93:80–83
203. Willis JS (1982) The mystery of the periodic arousal. In: Lyman P, Willis JS, Malan A, Wang LCH (eds) Hibernation and torpor in mammals and birds. Academic Press, New York, pp 92–101
204. Wingfield JC, Marler P (1988) Endocrine basis of communication in reproduction and aggression. In: Knobil E, Neill J (eds) The physiology of reproduction. Raven Press, New York, pp 1647–1677
205. Wolowyk MW (1997) Smooth muscle contractility and calcium channel density in hibernating and nonhibernating animals. Can J Physiol Pharmacol 68:68–70
206. Woods CP, Brigham RM (2004) The avian enigma: "hibernation" by common poorwills (*Phalaenoptilus nuttallii*). In: Barnes BM, Carey HV (eds) Life in the cold: evolution, mechanisms, adaptation and application. 12th International Hibernation Symposium. Institute of Arctic Biology, University of Alaska, Fairbanks, pp 129–138
207. Woods SC, Decke E, Vasselli JR (1974) Metabolic hormones and regulation of body weight. Psychol Rev 81:26–43
208. Woods SC, Porte D Jr (1978) The central nervous system, pancreatic hormones, feeding and obesity. Adv Metab Disorder 9:283–312
209. Wren AM et al (2001) Ghrelin causes hyperphagia and obesity in rats. Diabetes 50:2540–2547
210. Yacoe ME (1983) Maintenance of the pectoralis muscle during hibernation in the big brown bat, *Eptesicus fuscus*. J Comp Physiol 152B:97–104
211. Yenari M, Kitagawa K, Lyden P, Perez-Pinzon M (2008) Metabolic downregulation: a key to successful neuroprotection? Stroke 39:2910–2917
212. Zhang J, Kaasik K, Blackburn MR, Lee CC (2006) Constant darkness is a circadian metabolic signal in mammals. Nature 439:340–343
213. Zhao HW, Ross AP, Christian SL, Buchholz JN, Drew KL (2006) Decreased NR1 phosphorylation and decreased NMDAR function in hibernating Arctic ground squirrels. J Neurosci Res 84:291–298