Review

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Life in the cold: links between mammalian hibernation and longevity

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Abstract: The biological process of aging is the primary determinant of lifespan, but the factors that influence the rate of aging are not yet clearly understood and remain a challenging question. Mammals are characterized by >100-fold differences in maximal lifespan, influenced by relative variances in body mass and metabolic rate. Recent discoveries have identified long-lived mammalian species that deviate from the expected longevity quotient. A commonality among many long-lived species is the capacity to undergo metabolic rate depression, effectively re-programming normal metabolism in response to extreme environmental stress and enter states of torpor or hibernation. This stress tolerant phenotype often involves a reduction in overall metabolic rate to just 1–5% of the normal basal rate as well as activation of cytoprotective responses. At the cellular level, major energy savings are achieved via coordinated suppression of many ATP-expensive cell functions; e.g. global rates of protein synthesis are strongly reduced via inhibition of the insulin signaling axis. At the same time, various studies have shown activation of stress survival signaling during hibernation including up-regulation of protein chaperones, increased antioxidant defenses, and transcriptional activation of pro-survival signaling such as the FOXO and p53 pathways. Many similarities and parallels exist between hibernation phenotypes and different long-lived models, e.g. signal transduction pathways found to be commonly regulated during hibernation are also known to induce lifespan extension in animals such as *Drosophila melanogaster* and *Caenorhabditis elegans*. In this review, we highlight some of the molecular mechanisms that promote longevity in classic aging models *C. elegans*, *Drosophila*, and mice, while providing a comparative analysis to how they are regulated during mammalian hibernation.

Keywords: aging; insulin signaling metabolic depression; stress resistance; torpor.

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Introduction

Many mammals employ strategies of metabolic rate depression – entry into winter hibernation, summer aestivation, or daily torpor – to allow them to extend their survival chances under extreme environmental conditions (1, 2) Hibernation is perhaps the best known phenomenon and has been observed in eight different groups of mammals: the monotremes (echidna), marsupials (pygmy-possum), rodents (ground squirrels and others), bats (many species), insectivores (hedgehog), carnivores (black bear), elephant shrews, and primates (selected lemurs) (1, 3). For smaller hibernators such as bats, ground squirrels, and marmots, hibernation involves extended periods of deep torpor, during which core body temperature (Tb) falls to near ambient, interspersed with brief periods of arousal back to euthermia. Large hibernators such as black bears are an exception and show a different pattern in their metabolic adaptations, with body temperature cooling only to 30–36°C during torpor (4). The inhibition of thermogenesis, while having no significant cellular damage, makes hibernation one of the most complex and regulated forms of hypometabolism, requiring control at the physiological and biochemical level. Prior to hibernation, metabolic re-programming is initiated that includes hyperphagia in the late summer / early autumn, which results in massive weight gain due to increased fat storage in white adipose tissue (5). Animals then typically go through a number of "test drop" events of short torpor bouts at reduced body temperatures that appear to induce metabolic re-programming. Subsequently animals can initiate prolonged periods of torpor (days to weeks) with up to 95–99% reduction of basal

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metabolic rate as compared to the nonhibernating state, body temperatures that can fall to near 0°C, and metabolism switched over to a main reliance on lipid as the primary metabolic fuel for all organs (6). In addition to regulating metabolic fuel storage to support hypometabolism, hibernators are also faced with increased cellular stress during their torpor bouts. The decrease in respiration and heart rate during torpor creates an environment that is vulnerable to hypoxia/ischemia damage, whereas animals are also susceptible to oxidative stress during interbout arousals when metabolic rate and oxygen consumption increases massively to rewarm the animal back to euthermic conditions (7). As such, hibernators are incredible models to study the mammalian metabolic plasticity and stress resistance.

To date, our primary interest in hibernation has been geared towards understanding the regulation of transitions to/from the hypometabolic state and the cell preservation strategies that are needed to support long-term viability of the animal in torpor (7). These studies of natural torpor are not only important for understanding torpor/hibernation as biological phenomenon but also provide valuable information that could be used to for multiple biomedical applications including to extend the preservation time of organs for use in transplant medicine, or minimize degeneration of tissues (such as skeletal muscle) in patients experiencing prolonged inactivity. However, entry into a hypometabolic state can also be viewed as a strategy of lifespan extension that can potentially "suspend" aging. Metabolic rate typically correlates positively with body size in mammals and negatively with longevity but hibernating species are outliers in this relationship. This is supported by the extreme longevity displayed by various mammals that can either enter hibernation (bats, ground squirrels, lemurs, tenrecs, and marmots) or have the capability to depress their metabolism during stress (e.g. naked mole rats during chronic diet restriction) (8). Recent studies have shown that many hibernators live well past their expected lifespan as predicted from body size or euthermic metabolic rate (Table 1). Bats in particular exhibit extreme lifespans of 38–41 years, 9.8 times longer than expected based on their body size. In addition to bats, other long-lived hibernators include ground squirrels (~7 years), marmots (~20 years), and mouse lemurs (8–14 years) (Table 1) (9–14). Although non-hibernating rodents such as Eastern gray squirrels can also exhibit exceptional longevity, studies with Turkish hamsters have shown that animals that spend a higher portion of their life in hibernation have greater lifespans (15). This evidence suggests that there is a link between hibernation and longevity, and it is conceivable that the mechanisms that hibernators employ to survive during torpor could be linked to mechanisms that delay aging.

There is much interest in understanding the process of aging, and humans have long pursued interventions that can prolong lifespan and promote healthy aging. As animals get older, there is a persistent decline in intrinsic physiological function that occurs in a time-dependent manner; this is caused by an accumulation of cellular and tissue damage over time, resulting in increased mortality rate as time passes (16). Indeed, age is the primary risk factor for the development of many neurological and metabolic diseases, including cardiovascular disease, type-2 diabetes, Alzheimer's, and cancer (17–20). A number of theories on factors that influence aging have been proposed. These models stem from the idea that the human body's ability to repair cellular injuries declines over time, leading to the accumulation of errors and damage that affect metabolic processes and result in genetic instability and eventual pathological states (21).

While metabolic rate depression and stress resistance have been shown to be the fundamental mechanisms that are required to support hibernation, they are also two of the most common cellular processes that have been shown to directly influence aging in *Drosophila melanogaster* and nematode worms *Caenorhabditis elegans* (22–26). In the present review, we discuss recent advances in the field of hibernation across different mammalian species,

Table 1: Recorded lifespan of animal models that hibernate/undergo metabolic depression compared to mouse and rat models.

Species	Common name	Lifespan	Body mass, g
Myotis lucifugus	Little brown bat	34 years (wild) (11)	5.8
Heterocephalus glaber	Naked mole-rat	31 years (captivity) (95)	35
Marmota flaviventris	Yellow-bellied marmot	21.2 years (captivity) (14)	4295.0
Echinops telfairi	Lesser hedgehog tenrec	19 years (captivity) (14)	180
Microcebus murinus	Gray mouse lemur	18.2 years (captivity) (14)	64.8
Ictidomys tridecemlineatus	13-Lined ground squirrel	7.9 years (captivity) (14)	198.4
Mus musculus	House mouse	4 years (captivity) (12)	20.5
Rattus norvegicus	Norway rat	3.8 years (captivity) (13)	300

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highlighting mechanisms that regulate hibernation and their potential contribution to an extended lifespan.

Insulin signaling cascade in aging and hibernation

The insulin signaling cascade is the central hub that controls energy homeostasis within the cell, and functions to govern multiple downstream cellular processes including protein synthesis, glucose and lipid metabolism, mitogen response, autophagy, and more (27, 28). Downstream of the insulin receptor, the serine/threonine kinase Akt can stimulate cellular growth through the mammalian target of rapamycin (mTOR) axis during periods of nutrient abundance, or promote activation of the forkhead box O (FOXO) family of proteins to enhance survival during periods of low nutrients or stress. The insulin signaling pathway regulates a host of metabolic functions that are directly linked to aging and longevity; these include but are not limited to, the control of protein synthesis, autophagy, and transcriptional activation of stress resistance genes (Figure 1).

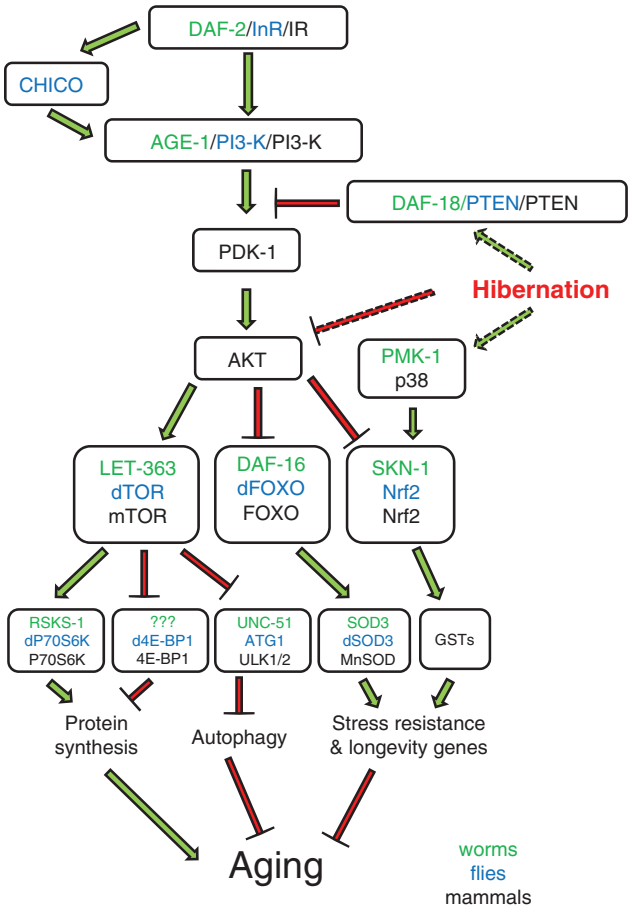

Figure 1: Regulation of the insulin signaling cascade in worms, flies, and mammals.
The conserved insulin signaling pathway regulates metabolic processes that are directly linked to aging that include protein synthesis, autophagy, and transcription of stress responsive and longevity genes regulated through FOXO and Nrf-2 transcription factors. During hibernation, the insulin signaling cascade is attenuated through inhibition of Akt kinase and activation of PTEN phosphatase; meanwhile, the p38 MAP kinase signaling cascade is activated as part of a stress response during hibernation.

Insulin signaling and aging

In studies across multiple species, there is considerable evidence that suggests that the insulin signaling pathway is prominently involved in the control of aging. Studies in *C. elegans* and *Drosophila* have provided invaluable models towards understanding the fundamental mechanisms of insulin signaling in aging, since the core insulin signaling pathway is highly conserved between invertebrates and mammals. In *C. elegans*, mutations to *daf-2* and *age-1*, the orthologues of insulin/insulin-like growth factor-1 (IGF-1) receptor (IGFR1) and phosphoinositide 3-kinase (PI3K), respectively, can double the lifespan of worms compared to the wild-type; extension of lifespan in both *daf-2* and *age-1* mutants is completely dependent on the functions of *daf-16* which encodes for the mammalian FOXO protein (29). DAF-16 is a transcription factor that is negatively regulated by the insulin-like signaling pathway in *C. elegans*, and is required for dauer formation during periods of unfavorable developmental conditions, as well as to mediate the stress resistance response throughout adulthood (30). Mutation in *daf-2* not only extends lifespan in *C. elegans*, it also confers increased resistance towards oxidative stress, mediated through the transcriptional activation of DAF-16 dependent stress resistances genes (31). Depletion of LET-363/TOR (target of rapamycin) kinase that functions downstream of IGF signaling can also extend lifespan (32). TOR is a highly conserved protein kinase that functions as a nutrient sensor throughout evolution, and is a central regulator of protein synthesis, cell growth, and metabolism (33). Interestingly, *daf-16* is not required for TOR mediated lifespan extension, suggesting that TOR could function downstream or independent of DAF-16 to regulate aging. In addition to regulating longevity, *daf-2*, *daf-16*, and *let-363/tor* have all been found to play a major role in dauer formation, a stress resistant quiescent state much like hibernation that relies on internal energy reserves for survival (32, 34, 35).

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Similar findings on the role of insulin signaling and aging have also been reported in flies and mice. Lifespan extension can be achieved through mutations to the *Drosophila* insulin-like receptor or the insulin receptor substrate CHICO, whereas dFOXO (*Drosophila* FOXO) activation can increase lifespan in a cell non-autonomous manner (22, 36). Mutations that directly inhibit the TOR pathway in *Drosophila* are also sufficient to cause lifespan extension (23). In mice, various mutations that impede insulin growth factor-1 (IGF-1) production have also been linked to increased longevity, mice exhibiting increased lifespan having reduced levels of circulating insulin along with increased insulin sensitivity (37). Inhibition of mammalian TOR via rapamycin feeding has been shown to increase lifespan by 14% in female and 9% in male mice (38). Overall, there is an overwhelming amount of evidence demonstrating that inhibition of different components of the insulin signaling pathway contribute to increased longevity, and this is a conserved response across many species.

Insulin signaling in hibernation

Hibernators undergo several physiological and biochemical changes during torpor, most notably, hibernators reduce their carbohydrate oxidation during torpor and shift towards a reliance on lipid based metabolism, utilizing their increased fat storage as the primary source of metabolic fuel (39, 40). To conserve metabolic fuel and maintain a hypometabolic state during torpor, hibernators must prioritize their energy utilization to prolong survival. One mechanism to minimize energy usage is to selectively turn off (or strongly turn down) non-essential cellular processes during torpor and minimize catabolic pathways that utilize ATP. These include the suppression of mitosis and cell proliferation, mitochondrial metabolism, transmembrane ion transport, global mRNA transcription, and protein biosynthesis (41–45).

Protein translation is one of the major energy expensive processes in the cell, utilizing up to 40% of total ATP production, with ~5 ATP equivalent required per peptide bond formed. Expectedly, protein synthesis was one of the first cellular processes show to be inhibited during hibernation, along with other ATP-consuming processes like transmembrane ion pumping and gene transcription (43, 44, 46). Protein synthesis in ground squirrels was actively suppressed in the brain, liver, and heart of ground squirrels during hibernation, with hibernating brain exhibiting only ~0.04% of the value in active squirrels (44). Importantly, it was shown that the suppression of protein synthesis observed during hibernation was independent of ambient temperature, which demonstrated that the reduction in protein synthesis was not simply a function of passive thermodynamic effects of the decrease in ambient temperature, but rather was a highly regulated process. The inhibition on protein synthesis is associated with a strong increase in phosphorylation of the eukaryotic initiation factor 2α (eIF2α) on serine 51 by ~6.5-fold, which inhibits the guanidine nucleotide exchange factor eIF2B and prevents GTP recycling between each round of translation initiation (47). The decrease in translation activity during hibernation is a direct result of disaggregation of ribosomes; ribosomal profiles during hibernation show a shift to a decrease in ribosomes in polysome fractions and an increase of monosome content, indicating a reduced abundance of active translational complexes (44, 48). Upstream of ribosome assembly, the translational complex is also positively regulated by p70S6K and negatively by 4E-BP1, both of which are downstream substrates of mTOR kinase as part of the insulin signaling cascade (49). Phosphorylation of p70S6K is required for its subsequent activation of the S6 small ribosomal subunit, whereas phosphorylation of 4E-BP1 prevents its inhibitory effect on eIF4E which initiates protein translation by recruiting the 40S ribosomal subunit to the 5′ end of the mRNA (49). In a nutrient rich state, insulin signaling activates mTOR through phosphorylation and inhibition of tuberous sclerosis 2 (TSC2) protein by Akt. Non-phosphorylated TSC2 forms an inhibitory complex with TSC1 that blocks the mTOR mediated phosphorylation of p70S6K and 4E-BP, which is required for an active translational complex (50). During hibernation in ground squirrels, Akt kinase activity has been shown to be reduced in multiple tissues including the brain, muscle, and liver by 40–60% (51–54). The decrease in Akt activation was recently shown to be linked to an increased catalytic activity of phosphatase and tensin homolog (PTEN) phosphatase, which controls the activation of the Akt upstream kinase phosphoinositide-dependent kinase (PDK)-1 (55). The decreased Akt activity is accompanied by a reduction of mTOR activation and subsequent decrease in phosphorylation of S6 ribosomal protein and 4E-BP1, establishing a state of protein synthesis inhibition during torpor (53, 54, 56).

Similar suppression of the insulin signaling pathway has also been shown for other hibernating species. Studies in bats showed that phosphorylation of Akt and mTOR was reduced by 61% and 48%, respectively, 3 months into hibernation. Reduction in insulin signaling activity

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in the skeletal muscles was linked to the resistive properties toward muscle atrophy during long term disuse (57). Although insulin signaling control via phosphorylation cascades is a conserved process, evolutionary adaptations at the genetic level have also recently been shown. Genome wide analysis in Brandt's bat (*Myotis brandtii*) which has a lifespan exceeding 40 years revealed unique amino acid substitutions in the transmembrane domain of IGF1R (homologue of *C. elegans* DAF-2) that were different compared to other mammals (human, mouse, cat, horse) but conserved in different bat species (little and big brown bats) (58). Although the exact function of these amino acid substitutions in IGF1 in bats remains to be determined, the evolved IGF1 axis in these bats is linked with changes in the expression levels of several insulin-signaling related genes, including increased expression of *foxo1* to levels that are similar to the increase observed between long-lived *GHR*-/- (growth hormone receptor) and wild-type mice (58). Furthermore, *M. brandtii* show similar levels of expression changes in 11 out of 15 genes that were uniquely identified in long-lived mice compared to the wild-type; these included decreases in the expression of genes within the insulin signaling pathway such as *igf1*, *egfr* (epidermal growth factor receptor), and *igfals* (insulin-like growth factor binding protein, acid labile subunit) (58).

In addition to ground squirrels and bats, a recent study has also shown that Akt and mTOR, along with other components of insulin signaling, were significantly reduced in hibernating bears in winter (January) compared to pre-hibernation (October) and post-hibernation (May) (59). It appears, then, that the suppression of insulin signaling pathway is a conserved mechanism across hibernator species, and that this is likely a common response to inhibit metabolic activities during hibernation. However, it is not yet clear if the reduction of insulin signaling observed in hibernators is directly linked to their extended lifespan as compared to other mammals of similar body size. Studies in invertebrates and mice have taken advantage of genetic manipulations to identify the insulin signaling pathway as a direct regulator of aging. Conversely, models such as hibernators allow us to understand the effects of environmental manipulations on metabolic adaptations and its potential link to longevity. Global suppression of the insulin signaling pathway triggered in hibernators mirrors the regulation patterns that produce a long-lived phenotype from worms to mammals. Although it remains to be determined how exactly insulin signaling contributes to hibernator lifespan, this provides a potential context in which the environmental effects on metabolic regulation could impact longevity.

Stress resistance and pro-survival signaling in aging and hibernation

While suppression of insulin signaling can contribute to longevity by inhibiting pro-growth processes such as protein synthesis via TOR regulation, the insulin signaling pathway is also integrated into the regulation of stress resistance and pro-survival transcription factors such as FOXO/DAF-16 and Nrf-2/SKN-1, both of which regulate oxidative stress and contributes to lifespan extension (Figure 1) (60–63). Oxidative stress and insulin signaling are tightly linked to one another; increases in oxidative stress can lead to activation of stress sensitive signaling pathways that include NF-kB, JNK/SAPK, and p38 MAPK, which can disrupt insulin signaling by phosphorylating the insulin receptor substrate-1 and interfere with downstream target activations. Meanwhile, a decrease in insulin signaling can result in altered cellular energy balance due to reduced glucose uptake, and this elevation of glucose levels has been shown to influence the redox state of mitochondria, which can increase reactive oxygen species (ROS) generation leading oxidative stress and apoptosis (64, 65).

ROS, stress resistance, and aging

The free radical theory of aging was first introduced by Harman in 1956, which postulated that accumulation of ROS molecules, byproducts of aerobic metabolism, was the primary cause of aging (66). ROS (e.g. superoxide, hydroxyl radical, and singlet oxygen) are reactive and damaging towards carbohydrates, lipid, protein, and DNA, and can lead to destructive tissue injuries when accumulation persists. Despite nearly 50 years of research dedicated to deciphering the effects of ROS in the cell, the role of oxidative stress damage on aging remains debated. Recent studies have shown that an increase in mitochondrial superoxide generation through treatment with the low level superoxide generator, paraquat, can significantly increase lifespan in worms, indicating beneficial effects of ROS molecules (67). In addition, ROS have been shown to act as a positive regulator of intracellular signaling, and are important for the functions of many physiological parameters (20). As mentioned earlier, lifespan extension through mutations of the insulin receptors require the downstream action of DAF-16/FOXO, which is a transcription factor that functions as a central regulator of the stress response and is conserved across most (or potentially all) species. DAF-16/FOXO is normally inactivated by Akt kinase during periods

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of nutrient abundance, via phosphorylation that causes cytoplasmic sequestration of the transcription factor (68). However, under conditions that diminish insulin signaling, phosphorylation of DAF-16/FOXO is reversed, leading to its nuclear inclusion and the subsequent transcriptional activation of several hundred target genes, with many of these genes providing a concerted beneficial effect aimed towards increased stress resistance and longevity (68). Interestingly, recent studies have shown that although stress resistance is observed in many long-lived phenotypes, it is not an essential prerequisite for longevity. Reduction of *sod* genes in *daf-2* mutants sensitizes the worms toward oxidative stress, but has no negative effect on their longevity, despite the fact that some of these mutants produced higher levels of mitochondrial superoxide compared to wild-type (69, 70). Similar findings have also been shown in worms under dietary restriction (DR), an intervention that increases lifespan across multiple species. DR promotes the expression of SOD and its activity, but deletion of the *sod* genes during DR has no effect on the positive benefits that DR has on lifespan (71). While many long-lived species demonstrate increased levels of stress resistance, studies in worms suggest that enhanced antioxidant enzyme activities do not appear to be required for a long-lived phenotype, and in some cases, the deletion of mitochondrial *sod*, resulting in increased intracellular ROS, can even increase lifespan (72).

In addition to DAF-16, the transcription factor SKN-1 (orthologue of mammalian Nrf-2) which functions as the primary regulator of phase 2 detoxification has also been shown to be required for the *daf-2* dependent increase in lifespan of *C. elegans* (62). Worms and flies that show increased activation of SKN-1/Nrf-2 transcriptional activities are longer-lived compared to wild-types (61, 62). SKN-1/Nrf-2 is activated in response to oxidative stress and promotes transcription of genes that function as redox regulators such as glutathione S-transferases, thioredoxins, heme oxidase and many others (63, 73). Although both DAF-16/FOXO and SKN-1/Nrf-2 are required to promote regulation of lifespan in response to insulin signaling, and are both activated by reduced insulin signaling, they are controlled by different regulators and induce transcription of different sets of downstream genes. Intriguingly, both DAF-16/FOXO and SKN-1/Nrf2 promote transcription of genes that function outside of their best known roles in ROS detoxification when activated by oxidative stress; these include regulation of metabolism by promoting fat storage and expression of lipid metabolic genes (74, 75). Although it seems reasonable that an increase in antioxidant defense can promote lifespan by reducing cellular damage by limiting ROS accumulation, recent findings have shown that key transcriptional regulators such as DAF-16/FOXO and SKN-1/Nrf-2 may provide additional mechanisms to regulate longevity in addition to their canonical detoxification roles.

Stress resistance and antioxidant response in hibernators

One of the characteristics of nearly all animals that enter into hypometabolic dormant states is the activation of antioxidant defenses that contribute to a stress resistance phenotype. Due to extreme changes in metabolic activities that take place during hibernation (and other forms of hypometabolism) along with fluctuations in oxygen consumption caused by apneic breathing, antioxidant responses and stress resistance play a crucial role in minimizing tissue damage when animals are faced with potential transient ischemia/reperfusion insult during entry and exit from hibernation. Even brief periods of ischemia/reperfusion can be fatal to oxygen sensitive tissues such as the brain and the heart (76). While animals are in torpor, constitutive activation of stress resistance is crucial to maintain cell viability over weeks of torpor; meanwhile, antioxidant defense plays a major role during hibernation arousal, which is driven by a rapid increase in oxygen uptake to fuel thermogenesis and, as a consequence, is a period of high vulnerability to oxyradical formation. Many studies have focused on characterizing the protective mechanisms that support hibernation in bats and ground squirrels. Little brown bats, *Myotis lucifugus*, have a maximum lifespan of ~34 years and show a significant reduction in hydrogen peroxide production by more than 50% in the brain, heart, and kidney compared to short tailed shrews (average lifespan ~2 years), despite showing similar levels of oxygen consumption. This reduction in free-radical production is partially attributed to ~2-fold higher SOD activities in bat heart, but could also be a product of increased mitochondria efficiencies in the little brown bat, marked by reduced free radical production per oxygen consumed (77). Tissues of *Myotis* bats also display increased resistance towards protein oxidation under acute oxidative stresses both *in vivo* and *in vitro*, with lower levels of protein carbonylation following oxidative stress compared to mice (78).

During ground squirrel hibernation, protein contents of catalase along with SOD1 and SOD2 are significantly elevated in brown adipose tissue during hibernation by 1.5- to 2-fold, with expression of catalase and SOD1 also up-regulated by the same magnitude in active animals

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when maintained in the cold (79). Interestingly, this general trend of increased antioxidant protein levels was only observed in the skeletal muscle, but not in the liver or the white adipose tissue. Similar findings were reported for the ground squirrels which show that up-regulation of intracellular antioxidant enzymes during hibernation are not globally observed but specific to individual tissues (76). These findings would suggest that different tissues likely experience varied levels of oxidative stress, which would influence the regulation pattern of antioxidant proteins during torpor. It has also been suggested that in the liver, the basal activity of SOD is significantly higher by ~4 to 6-fold compared to other tissues (kidney, brown adipose tissue, lung, heart, and spleen), and this elevated basal SOD activity could be sufficient to maintain antioxidant defense during hibernation without a need to upregulate SOD protein levels (79, 80). Although antioxidant responses play a vital role in ROS detoxification during entry and arousal from hibernation, the absence of a global regulation pattern in all tissues would suggest that either (1) selective up-regulation of antioxidant defense in a tissue-specific manner is sufficient to promote overall stress resistance in torpor, or (2) other stress responsive mechanisms could play a crucial role in maintaining the hibernation phenotype.

The transcription factor FoxO3a, like DAF-16, is activated in the absence of insulin signaling, and has been shown to be crucial in protection of quiescent cells from oxidative stress (81). In ground squirrels, expression of FoxO3a is strongly up-regulated in hibernation by 3 to 4.5-fold, and this is associated with increased FoxO3a transcriptional activation and up-regulation of its downstream genes (82). Although many of the genes induced by Foxo3a are directly involved in the ROS response (e.g. *prdx-3, catalase, MnSOD*), activation of Foxo3a also up-regulates the expression of genes that negatively regulate the cell cycle, these include *p27KIP1*, and *cyclin* G_2. Although not yet shown in hibernators, activation of FoxO3a can also function to promote DNA damage repair through the induction of *gadd45* (growth arrest and DNA damage-inducible protein 45), in addition to regulating skeletal and cardiac muscle atrophy and hypertrophy through expression of *atrogin-1*. Furthermore, FOXO proteins have also been shown to regulate other physiological functions via interactions with different co-activating proteins, these include regulation of adipogenesis and insulin sensitivity through PPARγ (peroxisome proliferator-activated receptor gamma), modulation of lipid metabolism through interactions with HNF4 (hepatocyte nuclear factor 4), and regulation of glucose metabolism through interactions with Dyrk1 (probable serine/threonine-protein kinase) and SHP (small heterodimer partner) (83–86). Although the role of FOXO proteins in hibernation has been partially characterized, much remains unknown about its potential regulation of lipid and carbohydrate metabolism during torpor, which could contribute to metabolic shifts that are crucial to maintain hibernation survival and promote longevity.

The transcription factor Nrf-2 is also a stress inducible protein that functions to enhance cytoprotection mediated through increased expression of phase 2 detoxification genes. Nrf-2 is the principal transcription factor that regulates the transcription of genes containing the antioxidant response element (ARE), with Nrf-2 null mice showing reduced levels of detoxification genes in addition to increased susceptibility towards toxic stressors (30, 87, 88) During hibernation, the protein expression of Nrf-2 is up-regulated in multiple tissues of ground squirrels including brown adipose, heart, liver, and skeletal muscle by 2 to 3.5-fold (46, 89). For example, up-regulation of Nrf-2 protein in hibernation has been linked to a significant increase in expression of its downstream target gene *heme oxygenase 1* (*ho1*) at both the mRNA and protein level in multiple tissues including the brain and the heart (90). HO1 catalyzes the first step of heme degradation, resulting in production of antioxidant molecules (e.g. biliverdin, bilirubin), and is crucial in maintaining redox homeostasis in aging rats (91, 92). Interestingly, recent studies have shown that increased levels of *ho1* mRNA are linked to insulin resistance and obesity in mice and humans, two physiological parameters that are essential for hibernators to enter torpor (93). The role of Nrf-2 in longevity was also recently characterized in the long-lived naked mole rats, *Heterocephalus glaber*. Although these mole rats do not undergo hibernation, they are highly resistant toward a wide range of cytotoxic stresses, undergo metabolic depression in response to nutrient deprivation, and are extremely long-lived with maximum lifespans approaching 30 years (8 times longer than mice with similar body size) (Table 1) (8, 94, 95). The basal expression of Nrf-2 in *H. glaber* is nearly 5-fold higher compared to short-lived mice. This is also correlated with a decrease in expression of the Nrf-2 negative regulator, Keap1, and 10–50 fold increases in Nrf-2 downstream targets: *hmox1, gsta1* (*glutathione S-transferase alpha 1*), and *nqo1* [*NQO1 NAD(P)H dehydrogenase, quinone 1*] (95, 96). Interestingly, amongst 10 different rodent species, levels of Nrf-2 DNA binding activity are significantly correlated with lifespan, suggesting Nrf-2 is a crucial regulator that positively promotes longevity (96).

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Environmental impacts on aging and longevity

Temperature is a major parameter that affects essentially all processes at the chemical and biological levels. A moderate change in environmental temperatures has been shown to significantly impact lifespan in many poikilotherms (97). In both *Drosophila* and *C. elegans*, it is generally established that the lifespan of animals varies inversely with temperature, with animals maintained at a lower temperature usually living significantly longer than those at a higher temperature (98, 99). This interaction between temperature and lifespan are less defined in homeotherms, as fluctuations in core body temperature can have serious and detrimental effects in warm-blooded animals. In mice, slight reductions of core body temperatures (0.2–0.5°C) have been associated with an increase in lifespan. Mice maintained on a caloric restriction diet that prolonged lifespan display both a lower resting oxygen consumption and body temperature (100). Transgenic mice that over-expressed the uncoupling protein 2 (UCP2) in the hypocretin neurons have also been shown to reduce the mice's core body temperature by 0.2–0.5°C, resulting in an average increase of lifespan by 12% in males and 20% in females while maintained on an ad libitum diet (101). These studies suggested that lowering of core body temperature in mammals can have a positive effect on lifespan, and may account for some of the anti-aging effects of caloric restriction.

Both temperature and nutrient intake have profound effects on lifespan across multiple species, and are both major factors that influence hibernation. Reduction of environmental temperature is a major stimulus that induces hibernation in ground squirrels. Mammalian hibernators are unique that their core body temperature can drop to <10% of normal levels without eliciting a hypothermic response (7, 39). This reduction in body temperature is a controlled process, with suppression of metabolic processes taking place before the reduction in core body temperature (102); while in torpor, thermoregulation is controlled by non-shivering thermogenesis functioning to maintain core body temperature above freezing (103). In addition to a lowered core body temperature, hibernators also undergo prolonged fasting during torpor, a response that may be comparable to caloric restriction which increases lifespan across multiple species. Interestingly, ground squirrels overexpress fibroblast growth factors 21 (FGF21) in the liver during hibernation (104). FGF21 is an endocrine hormone that is strongly induced during starvation in mice, a response that increases hepatic fatty acid oxidation, insulin sensitivity, and sensitizes mice into a torpor like state (105, 106). Transgenic mice that overexpress FGF21 in the hepatocyte have significant increases in their longevity, with median survival time increased by 36% compared to wild-type mice. It is possible that the combination of a decrease in metabolic rate, body temperature, and food intake in hibernators function to promote longevity through a mechanism that lowers oxidative damages accumulated by a reduced metabolic system, however, this may be an over simplistic interpretation as recent studies have raised questions regarding roles of ROS in aging (107). Alternatively, environmental and physiological changes during hibernation (i.e. ambient temperature, metabolic rate, and nutrient availability) may provide integrative stimuli that promote molecular changes in multiple cellular signaling pathways, which in combination, are crucial for torpor survival and longevity.

Perspective and conclusions

While research in the aging field, to date, has utilized impressive genetic models that have uncovered many fundamental mechanisms that regulate aging and longevity in a conserved manner, research in non-traditional models such as hibernators can provide new insights into how environmentally-induced metabolic adaptations could influence aging and longevity. Hibernators may provide an advantage over traditional aging models as they naturally induce a hypometabolic state that triggers regulatory responses in a number of cellular signaling pathways which produce a significant increase in maximum lifespan when genetically altered in *C. elegans* or *D. melanogaster*. While the effects of temperature on aging is well established in poikilotherms, hibernators could allow researchers to explore this relationship in mammals without the restriction of hypothermic related cellular damages that are experienced in mice models. The regulation of insulin signaling, stress resistance, and antioxidant defense are three examples of cellular processes that are directly linked to aging and are conserved among multiple organisms. One emerging field of research is the regulation of small non-coding RNAs (ncRNAs) and their potential roles on aging. MicroRNAs are an example of such ncRNAs that have been shown to be crucial in the regulation of stress-responsive adaptations including hibernation, and are prominently involved in the control of aging and longevity (108–110). Understanding the mechanisms of the hibernation response is important from a comparative point of view, since the molecular mechanisms that regulate

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torpor-mediated metabolic depression are likely conserved across other similar adaptive stress responses such as anoxia and hypoxia tolerance, aestivation, and dauer/diapause. However, the uniqueness of hibernation as an adaptation in mammals provides potential applications for biomedicine. In addition to its potential importance in aging and longevity, hibernators are great research models for (1) natural organ preservation, as they experience minimal tissue damage while maintained at body temperature just above freezing, and (2) insulin resistance, as they undergo reversible periods of insulin resistance and obesity without the detrimental effects seen in diabetic patients (6, 38). With rapid improvement and commercialization of genome sequencing technologies, global transcriptomic analyses are increasingly proving to be an invaluable tool for providing detailed genetic insights into the regulatory mechanisms of hibernation. Indeed, a recent studies of long-lived hibernating bats provided not only information on the transcriptional changes that are associated with hibernation, but also novel discoveries of unique nucleotide changes within the insulin signaling pathway that are evolutionary conserved in other long-lived bat species, and could contribute to the hibernation phenotype as well as to the exceptional lifespan (58). Future studies in the field of hibernation biochemistry will continue to teach us about the mechanisms of stress adaptive metabolic regulation and potentially identify novel regulatory pathways that are associated with long-lived phenotypes.

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