Genomic Insights into Bear Hibernation

Blair W. Perry[1], Heiko T. Jansen[2] Alexis N. Enstrom[1,3], and Joanna L. Kelley[1]

1. Department of Ecology and Evolutionary Biology, University of California Santa Cruz, Santa Cruz, CA 95064
2. Department of Integrative Physiology and Neuroscience, Washington State University, Pullman, WA, 99163, USA.
3. Biomolecular Engineering and Bioinformatics, University of California Santa Cruz, Santa Cruz, CA 95064

Blair W. Perry:
blperry@ucsc.edu
0000-0003-4905-7852

Heiko T. Jansen:
heiko@wsu.edu
0000-0003-0178-396X

Joanna Kelley:
jokelley@ucsc.edu
0000-0002-7731-605X

Alexis Enstrom
aenstorm@ucsc.edu
0009-0003-2511-1962

Corresponding author:
Joanna L. Kelley
E-mail: jokelley(at)ucsc.edu
Phone: +1-831-459-5358

Keywords (max 6)

Hibernation, gene regulation, insulin resistance, evolution, metabolism, functional genomics

Abstract (max 150 words)

Hibernation is a fascinating adaptation to food-scarce winters, characterized by significant physiological and behavioral changes, including fasting, inactivity, and insulin resistance. While hibernation is critical for the survival of many species, hibernation-related traits are often considered pathological in humans. Hibernation has been studied from a genomic perspective, especially with respect to transcription across multiple tissues. These studies have identified differential activity of signaling pathways related to metabolism, tissue protection, and other mechanisms likely underlying hibernation phenotypes. Bears, in particular, are an interesting model for physiological and genomic studies of hibernation due to their large size and unique mode of hibernation compared to other small mammalian hibernators. Investigating the intricate molecular mechanisms underlying bear hibernation may therefore provide insight into fundamental biological processes with potential translational implications for human health, particularly with respect to metabolic disorders such as Type II Diabetes. This review focuses on recent advances and outstanding questions related to the exploration of bear hibernation from a genomic perspective.

Introduction

Mammalian hibernation involves a complex suite of metabolic and physiological shifts that reduce energy expenditure and enable survival during prolonged periods of resource scarcity (9, 32, 58, 67, 81). While the nature and magnitude of physiological shifts that occur vary between mammalian hibernators, hibernation typically involves reductions in metabolic rate, body temperature, and activity. Many hibernators do not feed for the duration of hibernation and rely on the metabolism of stored fat to maintain physiological function (13, 81).

Hibernation and hibernation-like adaptations are present in diverse mammalian lineages, including rodents (48), bats (78), primates (12), and bears (67). Among mammalian hibernators, bears are unique given their large size and distinct mode of hibernation. During hibernation, bears experience characteristic reductions in metabolic rate and activity but have a more modest reduction in body temperature compared to small hibernators. Bears maintain this torpor-like state for the duration of hibernation and do not exhibit interbout arousals as seen in small hibernators (Figure 1a). Research on hibernation to date has focused primarily on small mammals such as ground squirrels, due in part to the feasibility of working with these animals in a controlled laboratory setting. During hibernation, ground squirrels experience extreme bouts of torpor, during which body temperature is reduced to near-environmental levels (37). These torpor bouts are interspersed with brief periods of arousal when metabolic rate and body temperature return to near-normal levels ((43), Figure 1a). These interbout arousals enable periodic resumption of immune function, transcription and translation, and general cellular, molecular, and physiological maintenance of critical tissues and biological processes (reviewed in (1)). In bears, these processes are thought to be maintained throughout hibernation, albeit at reduced levels (39).

Species of bears that hibernate enter a period of hyperphagia every fall when they can consume upwards of 20,000 kilocalories per day to increase fat stores before entering hibernation (Figure 1b; (67)). In hyperphagia bears can gain as much as 20-70% of their summer body weight, the majority of which results from increased adiposity (34, 36). Bears then enter hibernation, during which heart rates drop to as low as 10 beats per minute, body temperature drops by 4-7º C, and metabolic rates drop by as much as 75% (32, 65, 67). When hibernating, bears are largely inactive; however, circadian rhythms and metabolic processes continue to be expressed (39, 99). Bears also do not eat, urinate, or defecate during hibernation (32, 67). Lipolysis of fat stores serves as the primary source of energy production, and multiple tissues become insulin resistant, presumably to spare glucose for use by the brain (79). Following spring emergence from hibernation, bears regain insulin sensitivity and return to a “normal” metabolic and physiological state having typically depleted a large portion of stored fat (35). Bears gain lean mass in the spring (34), and in the fall the cycle of hyperphagia, hibernation, and active seasons begins again. Despite undergoing these extreme shifts in body mass, metabolism, and activity on an annual basis, bears do not experience any known metabolic disease or other detrimental effects.

The ability to hibernate is variable across bear species, such that some bears do not hibernate at all while others may hibernate for six months out of the year (Figure 1C). Three of the extant bear species (Asiatic black bear (*Ursus thibetanus*), American black bear (*Ursus americanus*), and brown bear (*Ursus arctos*) exhibit hibernation during the winter (67, 89). A clade of recently extinct cave bears are hypothesized to have hibernated (27, 74). The giant panda (*Ailuropoda melanoleuca*), spectacled bear (*Tremarctos ornatus*), sloth bear (*Melursus ursinus*), and sun bear (*Helarctos malanayus*) remain active year-round and do not exhibit any known hibernation-like adaptations. The polar bear (*Ursus maritimus*) represents a particularly interesting example of metabolic and physiological innovation. Polar bears diverged from brown bears an estimated 500,000 years ago (55), and have since undergone rapid morphological, behavioral, and physiological adaptation to novel challenges of the Arctic environment. Notably, male polar bears do not rely on months-long hibernation like brown bears, and instead remain active year-round (71, 72). However, female polar bears do retain some hibernation-like adaptations, as discussed further below.

The unique nature of bear hibernation makes it a particularly valuable system for investigating the extremes of physiological adaptation in large mammals and for gaining new perspectives on metabolic disease in humans. In this review, we highlight recent advances in understanding hibernation in bears using diverse genomic approaches and highlight major unanswered questions that future research should address.

Genomic resources for studying hibernation

Understanding the genomic basis and evolution of hibernation in bears necessitates high-quality genomic resources to serve as the foundation for analyses of gene expression, sequence evolution, and more. Genome assemblies are available for all eight extant bear species, though the completeness and quality of these assemblies vary widely (3). At the time of this review, annotated, near-chromosome-level genome assemblies are only available for the giant panda (*A. melanoleuca*; NCBI: GCF_002007445.2; (52)), brown bear (*U. arctos;* UrsArc2.0, NCBI: GCF_023065955.2; (3)) and American black bear (*U. americanus*; mUrsAme1.0, NCBI: GCA_024610735.1; (94)). Unannotated chromosome level or scaffold-/contig-level assemblies are currently available for the polar bear (*U. maritimus;* NCBI: GCF_017311325.1; (50)), spectacled bear (*T. ornatus*; NCBI: GCA_028551375.1; (18)), sun bear (*H. malayanus*; NCBI: GCA_028533245.1; (18)), and Asiatic black bear (*U. thibetanus spp.;* NCBI: GCA_009660055.1, GCA_014364545.1; (47)). The additional development of high-quality annotated reference genomes for all extant bear species will expand our ability to investigate broad evolutionary questions related to hibernation and bear biology. While not publicly available at the time of publication, genome assemblies for all extant bear species are currently in progress (*Beth Shapiro, personal communication*). Accompanying these genome assemblies are a growing number of functional and population genomic datasets with immense value for understanding hibernation and bear biology in general. This includes, for example, whole-genome resequencing of brown bear samples spanning much of their global range (14, 88), numerous RNA-sequencing datasets collected from various tissues of hibernating and active bears (77, 96), and methylation data (63).

Gene expression shifts associated with bear hibernation

Many aspects of bear hibernation resemble conditions experienced in a variety of human diseases (Figure 2). A better understanding of how bears endure and reverse these states may provide crucial insights and further the development of novel therapeutics. Numerous studies have characterized the seasonal shifts in gene expression profiles that accompany the metabolic and physiological shifts occurring seasonally. Gene ontology or signaling pathway over-representation analysis have been used to characterize transcription profiles and infer activity of known pathways. As highlighted below, many of the genes implicated in hibernation are involved in metabolic and cellular pathways that are widely conserved across diverse species (including humans). Determining how these processes have been co-opted in bears and other hibernators to achieve dramatic physiological shifts can broaden our understanding of the mechanisms of hibernation and the evolutionary processes leading to this complex adaptation. Currently, the breadth and depth at which different tissues have been studied in the context of bear hibernation vary widely. Below, we highlight major findings from recent transcriptomic studies that have advanced our understanding of bear hibernation and its translational potential for human health.

Adipose

Bears accumulate massive fat reserves during hyperphagia in preparation for hibernation and subsequently rely on the metabolism of this stored fuel as their primary energy source in hibernation (32, 79, 81). Bear adipose tissue also becomes insulin resistant upon entering hibernation (59, 73, 79, 89). In humans, massive weight gain and insulin resistance precede the onset of Type II Diabetes (T2D) (8, 41, 42, 44, 104). In contrast, bears do not develop the hyperglycemia of T2D. Following emergence from hibernation in the spring, bears naturally regain insulin sensitivity and maintain this sensitivity until entering hibernation the subsequent winter (79). Understanding the molecular mechanisms governing these annual, cyclical metabolic shifts in adipose tissue will, therefore, not only reveal central mechanisms that enable hibernation but may inform novel approaches to the treatment and prevention of human insulin resistance, T2D, and other metabolic and obesity-related diseases.

Seasonal shifts in adipose gene expression were examined by Shimozuru et al. 2016 (90) using quantitative real-time polymerase chain reaction (qRT-PCR) to analyze differential gene expression of select candidate genes involved in energy metabolism in Japanese black bears (*Ursus thibetanus japonicus*). The results revealed that genes involved in lipogenesis and fatty acid synthesis were upregulated during hyperphagia, likely bolstering fat accumulation and weight gain during this season. Furthermore, genes involved in glycolysis and lipogenesis were downregulated while several genes involved in fatty acid catabolism were upregulated, consistent with a shift to reliance on stored fat as the primary energy source.

In a subsequent study, Jansen et al. 2019 (40) used RNA-sequencing to assess seasonal shifts in gene expression in adipose of brown bears (*Ursus arctos*). This study revealed massive differential expression of genes in adipose tissue with 6,139 genes – roughly 20% of the genes in the brown bear genome – exhibiting differential expression between active and hibernation seasons. Additionally, 984 genes were differentially expressed during hyperphagia in adipose. Similar to the Shimozuru study, downregulated genes in hibernation adipose samples showed enrichment for roles in glycolysis and fatty acid synthesis, consistent with a shift from glucose to lipid metabolism as the main energy source during hibernation. This study further identified differential expression of genes within the insulin signaling pathway that likely contribute to the onset of insulin resistance. Multiple genes that had been previously implicated in insulin resistance in humans via genome-wide association studies were also differentially expressed (56).

Studies of isoform-level gene expression in bear adipose tissue have revealed additional transcriptional complexity underlying hibernation (75, 96). These studies identified major shifts in isoform usage (i.e., the relative expression of different isoforms produced by the same gene) for numerous genes that did not exhibit significant changes in overall gene-level expression and were thus not detected in prior analyses of hibernation expression. Like analyses of gene-level expression, adipose tissue exhibited the largest number of genes with significant differential isoform usage between seasons (75). Genes with significant differential isoform usage in adipose tissue included several with known roles in regulating insulin sensitivity (i.e., *GRB14*), highlighting the value of including analyses of isoform usage when dissecting molecular mechanisms underlying hibernation phenotypes.

Other factors such as endocrine hormones may also play important roles in affecting adipose gene expression and function. For example, circulating cortisol concentrations are elevated during hibernation in bears (16, 97). This coincides with reductions in several key metabolic mediators in adipose including AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), and peroxisome proliferator-activated receptor alpha (PPARα) (97). As AMPK is normally elevated under conditions of energy deficit (33), this suggests that additional mechanisms may potentially induce insulin resistance in adipose of hibernating bears.

Liver

The liver also plays a major role in modulating metabolism and maintaining homeostasis within hibernation. Glucose production via gluconeogenesis increases in the liver of hibernating bears, which acts in conjunction with the shift towards fat catabolism as the dominant energy source (22, 89). Investigating the molecular mechanisms underlying metabolic shifts in the liver of hibernating bears may, therefore, inform our understanding of liver dysfunction in humans with obesity or other metabolic diseases.

Increased gluconeogenesis during hibernation has been investigated through multiple studies of hepatic gene expression in hibernating bears using microarrays (23, 25) qRT-PCR (89), and high-throughput RNA-sequencing (40). These studies identified both shared and unique signatures of gene expression associated with gluconeogenesis. For example, while early stages of gluconeogenesis are supported by upregulation of related genes in both black bears and brown bears, several major enzymes are differentially expressed in one species but not the other (40). This may indicate species-specific variation in hibernation mechanisms across bear species, although additional investigation using more comparable experimental designs and RNA-sequencing approaches are necessary to test this hypothesis.

In Jansen et al. 2019 (40) a total of 5,010 differentially expressed genes were identified between liver of hibernating and non-hibernating brown bears, indicating a considerable degree of differential regulation beyond genes underlying gluconeogenesis. Interestingly, comparatively few genes involved in the insulin signaling pathway were differentially expressed in liver compared to expression responses observed in adipose and muscle, indicating tissue-specificity in seasonal modulation of insulin sensitivity. In contrast to expression shifts observed in adipose tissue, liver only exhibited three significantly differentially expressed genes between the active and hyperphagia seasons. This finding suggests that liver does not experience hyperphagia-specific shifts in activity and that major shifts in liver expression and metabolism occur closer to the onset of hibernation.

Studies have also found evidence for the upregulation of tissue-protective mechanisms in hibernating bear liver. For example, genes involved in fatty acid metabolism are upregulated in hibernation (40), including *fatty-acid binding protein*-1 (*FABP1*) which may serve a protective function (100). Additionally, many differentially expressed genes in bear liver appear to underlie increased protein biosynthesis. Initially identified in microarray studies of black bear gene expression (22, 26) and subsequently supported by RNA-sequencing in brown bear (40), the upregulation of protein production in liver is likely involved in the maintenance and preservation of tissue during fasting. This contrasts with small mammalian hibernators in which protein synthesis is broadly decreased in hibernation (108), highlighting another unique feature of bear hibernation that warrants further investigation.

Heart

In hibernating bears, heart rate is reduced to as few as 10-16 beats per minute (65). Despite maintaining this low heart rate for months out of the year, bears do not experience congestive heart failure, cardiac chamber dilation, or other conditions typically associated with bradycardia (4, 65). Brown bears also tolerate elevated cholesterol levels in hibernation with no long-term atherosclerosis or other harmful consequences (2). Understanding the molecular mechanisms that protect heart tissue and function in hibernating bears may identify new biomolecules or therapeutic targets for the prevention and treatment of multiple human heart conditions and diseases.

Early investigations into cardiac gene expression in hibernating bears identified shifts in gene and isoform expression for a small number of candidate genes that likely play important roles in modulation of cardiac muscle structure and function during hibernation (4, 66). For example, Nelson et al. 2008 identified an increase in the abundance of a particular isoform of *titin*, a large gene that encodes structural proteins found in muscle (54), during hibernation. This change in isoform usage may contribute to stiffness of the ventricles, thus helping to prevent chamber dilation in hibernating bears (66). A subsequent RNA-sequencing study showed upregulation of genes involved in protein biosynthesis and downregulation of genes involved in amino acid catabolism that together may act to prevent cardiac muscle atrophy when hibernating (22). This contrasts with non-hibernating mammals in which starvation-induced atrophy of cardiac tissue is driven largely by a reduction in protein synthesis (86).

Skeletal Muscle

Prolonged disuse of muscle typically results in tissue atrophy and loss of muscle mass (5). In humans, muscle atrophy is a major concern for individuals with limited mobility due to prolonged hospitalization, injury, or other conditions (21). The most effective current approaches to both prevent and treat muscle atrophy in humans typically involve increased physical activity which may not be feasible for many patients (84). Despite pronounced inactivity during hibernation, bears are resistant to muscle atrophy and exhibit comparatively minor decreases in muscle mass and strength than would be expected for other mammals experiencing similar inactivity (31). This absence of muscle loss in hibernation appears to occur independently of nervous innervation (53). Understanding the genes and pathways underlying muscle preservation in hibernating bears is a promising avenue for discovering novel regulators of muscle function. This may be particularly relevant for the treatment of age-related muscle loss and disuse atrophy in humans.

Early microarray studies implicated increased expression of genes involved in protein biosynthesis as a major mechanism underlying the preservation of skeletal muscle mass in hibernating black bears (26). Subsequent transcriptomic and proteomic studies have found similar results in brown bears (40, 62). Of these, *pyruvate dehydrogenase kinase 4* (*PDK4*) and *serpin family F member 1* (*SERPINF1*) were identified as potential therapeutic targets. The upregulation of *PDK4* in hibernation likely contributes to minimizing muscle degradation (45), while *SERPINF1* downregulation likely contributes to the suppression of pathways that exacerbate muscle wasting (62). However, the exact mechanism by which *SERPINF1* affects skeletal muscle remains unknown (62). Consistent with the fact that bears remain physically active until the onset of hibernation, Jansen et al. 2019 found no differentially expressed genes between active and hyperphagia seasons in muscle (40). Serum also plays an important role in maintaining muscle viability and has been shown to promote growth of human myoblasts (10). The mechanisms through which these serum proteins alter/affect transcription remain to be fully elucidated.

Other studies have investigated oxidative stress as a potential pathway to study treatments for muscle atrophy (109). Oxidative stress, which is known to be a contributor to muscle atrophy, is effectively managed by hibernating brown bears and linked to their resistance to muscle atrophy (11). Hibernating bears have an upregulation of cold-inducible proteins such as CIRBP and RBM3, which are believed to help preserve muscle mass and mitigate oxidative damage (11). They also experience a downregulation in mitochondrial electron transport chain components and antioxidant enzymes, which suggests decreased reactive oxygen species (ROS) production in the skeletal muscle (11). An increase in the expression of cold-inducible proteins and minimal oxidative stress in brown bears play a critical role in preserving skeletal muscle (11). Muscle tissue in hibernating bears also is metabolically suppressed and insulin resistant (15, 79). Various genes involved in insulin signaling and related pathways have been found to be differentially expressed in hibernating muscle tissue (40, 62). Further investigations into the response of skeletal muscle oxidative stress and metabolism may provide potential insights into protecting against muscle atrophy in non-hibernating mammals.

Bone

Hibernating bears exhibit no significant loss of bone mass or strength despite prolonged inactivity (17, 60). Cortical porosity does not increase in hibernation as it would in humans under similar levels of inactivity, making bears a powerful model for discovering methods to prevent and potentially treat osteoporosis (60). Relatively few studies have investigated differential gene expression in bone tissue during hibernation. Fedorov et al. 2015 (24) identified the upregulation of genes with known roles in bone anabolic processes and downregulation of others involved in bone resorption and apoptosis. A subsequent study found similar patterns of downregulation in genes involved in the breakdown of bone, but did not detect differential expression of genes involved in bone formation (29). Together, these studies suggest that bone preservation during hibernation may be driven primarily by the reduction of the destructive processes that typically underlie disuse atrophy of bone tissue in humans. Further work is needed to clarify whether the upregulation of bone formation processes play a major role as well.

Kidney

Because the energy and water source for hibernation is fat (i.e., oxidation of fat produces carbon dioxide, ATP, and water), this provides for a nearly perfect enclosed system, eliminating the need to drink or urinate. However, even though they may not urinate for months, hibernating bears do not experience inflammation or kidney tissue damage (69, 92). Bear hibernation may, therefore, inform new approaches to treat chronic kidney disease and other renal diseases in humans (93). Renal gene expression associated with hibernation has only been investigated in a single study which analyzed gene expression in American black bear kidney samples collected during the fall (preceding hibernation) and early spring (immediately following hibernation; (92)). A relatively small number of differentially expressed genes (169 total) were detected in the kidney compared to the several thousand detected in other metabolic tissues in the studies described above. Consistent with the lack of notable inflammation in hibernating bear kidneys, this study detected the upregulation of several anti-inflammatory genes, including *SOCS2, CISH, and SERPINC1*, and did not detect upregulation of known kidney damage marker genes (92). Further investigation of bear renal gene expression that includes samples collected from mid-hibernation may provide additional insight into unique patterns of gene expression associated with renal function and maintenance.

Regulatory mechanisms driving hibernation gene expression

Understanding the biological and environmental signals that regulate hibernation signaling cascades will provide insight into the factors that coordinate large-scale, multi-gene gene expression shifts into and out of hibernation. Several recent studies have begun to tackle this through experimental manipulation of hibernation physiology in vivo and in vitro. By feeding bears enough of a glucose solution in mid-hibernation to replace the minimal metabolic cost of hibernation, recent studies tested whether nutrient intake was sufficient to reverse hibernation phenotypes and differential gene expression (38, 76). These experiments showed that mid-hibernation feeding stimulated only partial recovery of insulin sensitivity, heart rate, and other physiological metrics of hibernation (38). Similarly, analyses of gene expression showed that only a subset of hibernation-specific gene expression was reversed after feeding in adipose, liver, and muscle tissue (76). Additionally, inferences of upstream regulatory molecule activity implicated the transcription factor PPARG as a potential key regulator of “reversed” genes in these three tissues. Broadly, these results suggested that nutrient intake is an important signal for modulating hibernation physiology, but that other seasonal factors likely also play critical roles.

Multiple studies have implicated bear blood serum factors as critical regulators of diverse hibernation-associated processes in various tissues and bear species. Leveraging brown bear pre-adipocyte cell culture, a recent study tested the roles of circulating serum proteins in modulating cellular and gene expression phenotypes between active and hibernation seasons (87). Cells collected from hibernating bears that were stimulated with blood serum from the active season exhibited shifts in gene expression resembling expression profiles of active season cells, indicating the presence of regulatory signals in the serum. Proteomic analysis of active and hibernation blood serum identified eight proteins that were differentially abundant between seasons and thus likely involved in the stimulatory action of blood serum. Several of these proteins (i.e., IGF-1, IGFALS, and IGFBP-2) are known components of insulin signaling and related pathways. Additional work is needed to dissect the specific role(s) of these candidate proteins in regulating differential gene expression in adipose tissue. Blood serum has also been implicated in regulating the preservation of bone in Japanese black bears (*Ursus thibetanus japonicus*). Specifically, culture medium containing blood serum from hibernating bears was shown to inhibit the differentiation of peripheral blood mononuclear cells into osteoclasts, thus reducing the breakdown of bone tissue (64). Hibernating bear serum (and temperature) was also recently shown to reduce mitochondrial respiration in cultured bear adipocytes, indicating an important role in regulating metabolic suppression during hibernation (19). These studies collectively indicate that important signaling molecules are present in hibernating bear serum and that these molecules contribute to the regulation of diverse physiological and metabolic shifts. Future studies should continue to dissect compositional differences between active and hibernating bear blood serum and validate the roles of candidate signaling molecules in driving differential expression in multiple tissues. Such work is valuable for both rapidly advancing our understanding of high-level regulatory mechanisms of hibernation and identifying key molecules that may elicit similar biological responses in humans (19, 64).

Circadian control of gene expression plays diverse roles in the regulation of metabolism, homeostasis, and gene expression across multiple tissues (85). In small mammalian hibernators, circadian rhythmicity is largely lost during hibernation (107). Bears, however, retain circadian rhythmicity (39, 103), suggesting that circadian control of gene expression may play a uniquely important role in retaining metabolic function during bear hibernation. Recent studies on circadian gene regulation in bears have begun to make links between shifts in cellular circadian rhythms, gene expression, and metabolism, bringing us closer to understanding the significance of circadian gene regulation in bear hibernation (99). Observations that circadian rhythms are disrupted in diabetes (30) suggests this is a fruitful area of research.

microRNAs are small non-coding RNAs capable of regulating post-transcriptional gene products through either transcript degradation or repression of translation (83). In humans, microRNAs play major roles in the regulation of metabolism, and, accordingly, are often associated with metabolic dysfunction and disease (83). In bears, microRNAs have been implicated in the regulation of genes involved in metabolism and muscle maintenance in hibernating brown bears (57), as well as genes involved in metabolism in the liver of hibernating Japanese black bears (70). Investigating the regulatory roles of microRNAs across a greater diversity of tissues and species will shed additional light on the regulatory complexity that drives key hibernation phenotypes in bears.

Another underexplored area is sex-specific gene expression between hibernation and active seasons. To date, multiple studies including samples from both male and female bears have shown that gene expression patterns tend to cluster based on both season and sex (40, 87). These studies controlled for the effect of sex while investigating seasonal changes in gene expression but did not investigate whether this signature of sex-specific expression may be indicative of differences in physiology and metabolism. Investigating female-specific gene expression shifts during hibernation may be particularly interesting given that pregnant females give birth and nurse cubs during hibernation, and thus likely experience unique or elevated metabolic and physiological demands compared to males. Studies focused on sex-specific gene expression in hibernating bears will provide insight into sex-specific nuances in hibernation physiology.

Insight into the evolution of hibernation in bears and beyond

The presence of hibernation and torpor throughout the mammalian phylogeny, as well as the existence of similar forms of adaptive metabolic depression in other groups (i.e., brumation in reptiles and diapause in insects), raises interesting questions about whether hibernation is an ancestral trait that has been retained in some lineages and lost in others, or a common solution to seasonality that has independently evolved multiple times throughout the tree of life. In support of both possibilities, recent evidence suggests that torpor/hibernation may have evolved in a mammalian predecessor during the Triassic period (approximately 250 million years ago) in the seasonal Antarctic environment (106). Indeed, comparative studies have identified similar mechanisms underlying hibernation in diverse groups, ranging from the level of genes (105) to signaling pathways (98). While these findings may suggest a common evolutionary origin of hibernation, the alternative hypothesis that hibernation has arisen through convergence is yet to be conclusively rejected. As we continue to accumulate data and knowledge of hibernation across the tree of life, studies that more formally and explicitly test these hypotheses will become increasingly feasible and valuable.

Polar bears present unique opportunities to investigate genes underlying metabolic and physiological adaptation. Despite having diverged an estimated 500,000 years ago (Figure 1) and retaining high levels of synteny with brown bears (3), polar bears have undergone rapid and substantial morphological, behavioral, and physiological adaptation to the unique challenges of the Arctic. Polar bears are primarily carnivorous, consume a high fat diet, and leverage sea ice to feed during winter on adult seals or during the early spring on neonates (71). Additionally, hibernation is facultative in polar bears depending on their level of activity (71, 72). For example, adult males will lay down and reach hibernation-like daily energy expenditures during prolonged periods of fasting on land (72). Conversely, smaller subadult and female bears will often remain active and forage during periods on land, thus maintaining higher levels of daily energy expenditure (72, 82). Like brown bears, female polar bears will also den during the winter to give birth and are thought to experience reductions in activity and metabolism during this time comparable to hibernation (91). Several comparative and evolutionary genomic studies have identified genes involved in adipose tissue function, metabolism, and diet that have undergone rapid evolution in the polar bear lineage (55, 61, 80). However, a lack of functional genomic studies in polar bears constrains our understanding of seasonal gene expression shifts and limits comparisons with other bear species. Additional physiological and functional genomic studies of polar bears are needed to shed light on the mechanisms underlying their unique physiological and metabolic adaptations.

Polar bear and brown bear histories have been intertwined even after their relatively recent divergence. There is evidence of multiple unidirectional admixture events from polar bears into brown bears, including independent admixture events in Alaska, Ireland, and Japan approximately 15,000 years ago (7), and a much older 100,000 years ago admixture event that is present in genomes of all living brown bears (101). The evidence for unidirectional gene flow suggests that brown bear alleles are highly deleterious in polar bear genomes while the opposite is likely not true (6, 7). There is evidence that admixture from polar bears into brown bears from the Admiralty, Baranof, and Chichagof islands in the Alexander Archipelago of Alaska (ABC brown bears) has led to salt tolerant genes from polar bears to be present in ABC brown bears (61). The extent to which admixture is driving unique adaptations in specific populations of brown bears and/or polar bears (6, 7, 61) is a current research area and will benefit from additional genomes from extant and extinct populations.

Given that polar bears and brown bears can still readily hybridize, there is an expectation that climate change will lead to increased opportunities for admixture (51, 61). Given that Arctic environments are experiencing impacts of climate change at a faster/more significant rate than more temperate regions, polar bears will likely spend more time inhabiting areas overlapping with brown bears. Evidence of interbreeding between brown and polar bears raises interesting possibilities to explore adaptations in diet and other metabolic functions. Future efforts to understand the physiological and ecological impacts of climate change on hibernating bear species may provide valuable insight into conservation decisions.

In addition to questions about the evolutionary origins of hibernation, it is interesting to explore whether hibernation itself impacts the evolution and genome biology of hibernating species. For example, prolonged reductions in metabolism and spermatogenesis during hibernation may be expected to reduce per-generation mutation rates in hibernating bears and result in less male mutation bias than related hibernators (102). Interestingly, Wang et al. 2022 found no significant difference in per-generation mutation rate or male-bias in brown bears compared to the non-hibernating panda (102). Another study showed that hibernation results in reduced telomere shortening in black bears, thus potentially reducing the rate and impact of cellular aging (46).

Conclusions

The field of hibernation biology is poised for significant breakthroughs. Hibernation is accompanied by massive changes in gene expression in all tissues investigated. Significant progress has been made in understanding the genes, regulation, and evolution of hibernation through the application of diverse genomic approaches in a limited number of tissues. Genomic resources and datasets continue to accumulate for hibernating and non-hibernating bear species, enabling new avenues of research. There is an accumulating wealth of high-quality genomes, as well as ancient and contemporary population genetic samples. Moving forward, an emphasis on single-cell genomics, in vitro system development, and analysis of transition periods will greatly benefit our understanding of the evolution and mechanisms of hibernation and could result in important translational advances in biomedicine.

Single-cell analyses and spatial profiling

Hibernation-related transcriptional changes have been investigated from a whole tissue perspective through bulk microarray and bulk RNA-seq. Single cell genomics will be informative to tease-apart cell-type specific hibernation signatures that may be obscured in bulk tissue studies. By developing tissue and cell-specific expression profiles, it will be possible to develop the appropriate probe sets for spatial profiling (*in situ* RNA visualization) on formalin-fixed paraffin-embedded (FFPE) and cryopreserved tissues to determine the spatial context for expression changes.

in vitro systems

Given the extreme physiology of hibernation, many tissues and organ systems in addition to those discussed above are likely to exhibit adaptations to preserve tissue form and function in the presence of reduced metabolism, prolonged inactivity, and fasting. A major challenge of studying any tissue in bears, and especially deep internal tissues, is collecting a sufficient amount and number of samples while reducing, or ideally avoiding, lethal sampling. Multiple bear cell types have been successfully cultured in vitro to date, including pre-adipocytes (28), fibroblasts (99), peripheral blood mononuclear cells (64). The development of additional cell culture, organoid, and induced pluripotent stem cell systems for diverse bear tissues and multiple species will provide substantially greater access and experimental control for dissecting the mechanisms of hibernation, while reducing the need for sampling live animals.

Transitional periods

To date, nearly all genomic studies of hibernation have sampled bears during the middle of hibernation and active seasons to target the most contrasting physiological states (i.e., (26, 29, 40)). While these studies have provided insight into the fundamental differences between hibernating and non-hibernating bears, much remains unknown about the mechanisms, timing, and regulation of the transitional periods in which bears enter or emerge from hibernation. A complex interplay between environmental (i.e., photoperiod) and endogenous (i.e., circadian rhythm) cues are thought to trigger the onset and emergence from hibernation (20). For example, Nelson et al. showed that black bears will not enter hibernation during the summer when deprived of food, indicating an integral role of day length in controlling when bears hibernate (68). However, the underlying molecular mechanisms that contribute and/or respond to these complex cues remain largely unstudied and unknown. Additionally, physiological transitions likely occur throughout hibernation (38, 95) but are yet to be investigated in terms of molecular mechanisms and gene expression. Understanding the molecular, cellular, and physiological shifts that occur during these transitional periods will not only provide a more comprehensive understanding of hibernation but may reveal additional insight into aspects of hibernation that may be valuable for translational biomedical research. For example, identifying both the signals and molecular mechanisms that enable bears to regain normal insulin sensitivity at the conclusion of hibernation may reveal new molecular targets to aid in the reversal of insulin resistance in human patients. Filling these gaps in our understanding of transitional periods is, therefore, an extremely valuable and exciting direction for future studies to prioritize.

Figures

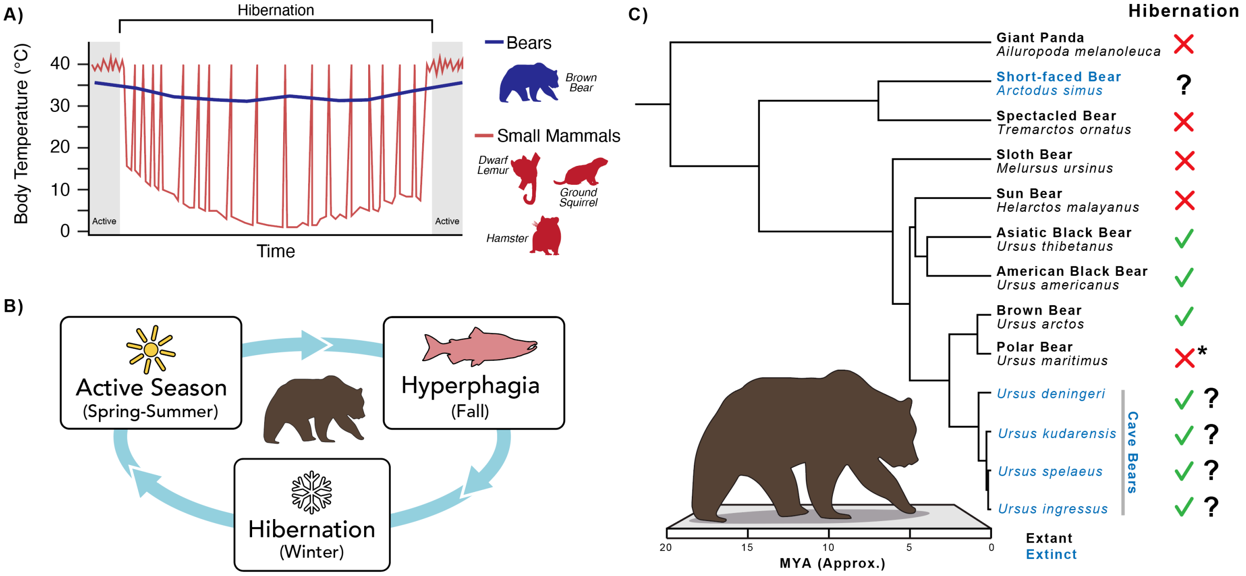

Figure 1. A) Cartoon schematic of body temperature in hibernation for bears (blue) and small mammalian hibernators (red). B) A year in the life of a hibernating bear is characterized by an active season during the spring and summer, hyperphagia in the fall, and hibernation or denning during the winter. C) Phylogeny of Ursidae containing both extant (black) and extinct (blue) lineages from TimeTree (49). To the right of the tree, symbols indicate whether a particular lineage hibernates (✓) or not (X). Question marks indicate uncertainty about hibernation capacity. Polar bears, marked with an asterisk, appear to exhibit facultative hibernation as discussed in the main text.

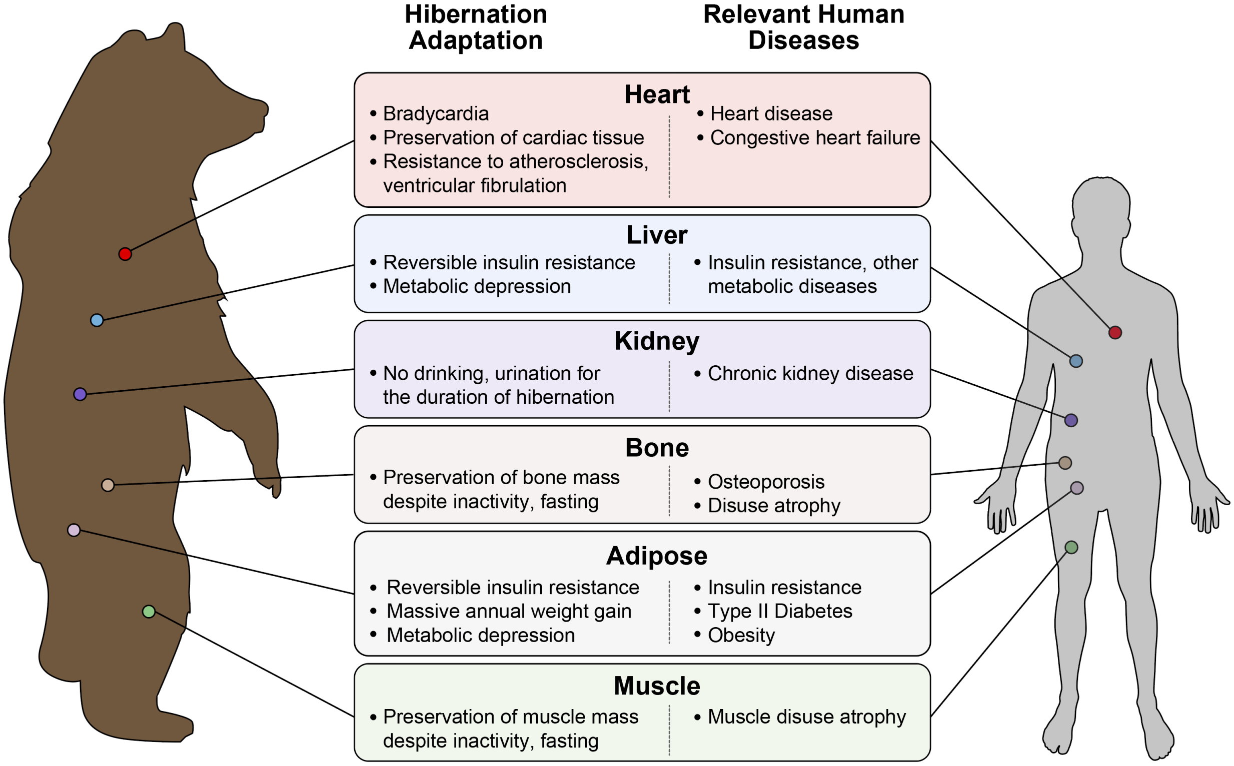

Figure 2. Aspects of bear hibernation with relevance for preventing and treating human diseases. Tissue and organ-level features of hibernation are highlighted on the left, with examples of relevant or parallel human diseases listed on the right.

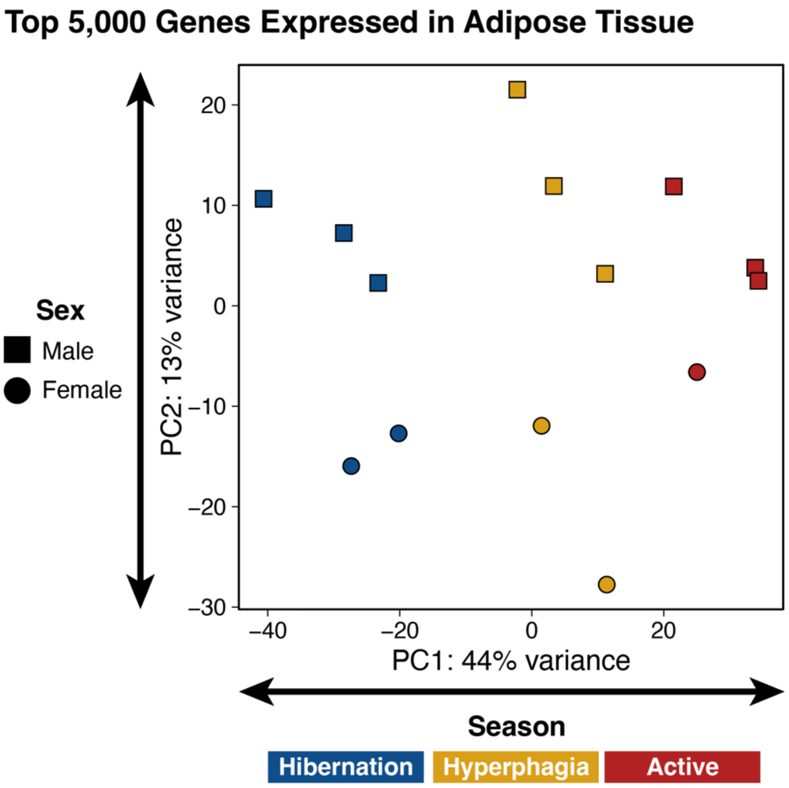

Figure 3. Principal component analysis (PCA) analysis of the top 5,000 genes expressed in adipose tissue in brown bears sampled during hibernation (blue), hyperphagia (yellow), and active season (red). PC 1 separates samples by season, while sex separates on PC2 (males: squares, females: circles). Expression data taken from Jansen et al. 2019 (40) and mapped against the brown bear genome assembly UrsArc2.0.

Terms and Definitions List

Admixture: the exchange of genetic material between divergent lineages

Circadian: natural rhythms that cycle roughly every 24-hours to control behavior, physiology, and more

Hyperphagia: physiological state during which bears consume massive quantities of food to gain mass and adipose tissue in preparation for hibernation.

Insulin resistance: a physiological state in which cells become unresponsive to insulin and do not uptake blood glucose.

Isoform: alternative transcripts and protein products produced by a single gene

Isoform usage: relative expression of the different isoforms produced by a gene

Gene Ontology: database of terms describing known functions and products of genes

Gluconeogenesis: metabolic process that results in the biosynthesis of glucose

Glycolysis: metabolic process that breaks down glucose to produce energy

Microarray: a technique to measure gene expression using probes for a pre-determined set of known genes

Oxidative Stress: an excess of reactive oxygen species that can result in damage to cells, proteins, and/or DNA

Quantitative real-time polymerase chain reaction (qRT-PCR): technique to measure the expression level of specific genes with high sensitivity

Serum: fluid that remains after blood cells and clotting factors are removed from blood

Torpor: physiological state in which metabolism, body temperature, and activity are decreased to conserve energy lasting hours to weeks

Literature Cited

1. Andrews MT. 2019. Molecular interactions underpinning the phenotype of hibernation in mammals. *Journal of Experimental Biology*. 222(2):jeb160606
2. Arinell K, Sahdo B, Evans AL, Arnemo JM, Baandrup U, Fröbert O. 2012. Brown bears (Ursus arctos) seem resistant to atherosclerosis despite highly elevated plasma lipids during hibernation and active state. *Clin Transl Sci*. 5(3):269–72
3. Armstrong EE, Perry BW, Huang Y, Garimella K V, Jansen HT, et al. 2022. A beary good genome: Haplotype-resolved, chromosome-level assembly of the brown bear (Ursus arctos). *Genome Biol Evol*. 14(9):evac125
4. Barrows ND, Nelson OL, Robbins CT, Rourke BC. 2011. Increased cardiac alpha-myosin heavy chain in left atria and decreased myocardial insulin-like growth factor (Igf-I) expression accompany low heart rate in hibernating grizzly bears. *Physiological and Biochemical Zoology*. 84(1):1–17
5. Brooks NE, Myburgh KH. 2014. Skeletal muscle wasting with disuse atrophy is multi-dimensional: the response and interaction of myonuclei, satellite cells and signaling pathways. *Front Physiol*. 5:99
6. Cahill JA, Heintzman PD, Harris K, Teasdale MD, Kapp J, et al. 2018. Genomic evidence of widespread admixture from polar bears into brown bears during the last ice age. *Mol Biol Evol*. 35(5):1120–29
7. Cahill JA, Stirling I, Kistler L, Salamzade R, Ersmark E, et al. 2015. Genomic evidence of geographically widespread effect of gene flow from polar bears into brown bears. *Mol Ecol*. 24(6):1205–17
8. Cahill Jr G. 1970. Metabolic adaptation to prolonged starvation in man. *Nord Med*. 83:89
9. Carey H V, Andrews MT, Martin SL. 2003. Mammalian Hibernation: Cellular and Molecular Responsesto Depressed Metabolism and Low Temperature. *Physiology Review*. 83:1153–81
10. Chanon S, Chazarin B, Toubhans B, Durand C, Chery I, et al. 2018. Proteolysis inhibition by hibernating bear serum leads to increased protein content in human muscle cells. *Sci Rep*. 8(1):1–10
11. Chazarin B, Storey KB, Ziemianin A, Chanon S, Plumel M, et al. 2019. Metabolic reprogramming involving glycolysis in the hibernating brown bear skeletal muscle. *Front Zool*. 16:1–21
12. Dausmann KH, Glos J, Ganzhorn JU, Heldmaier G. 2004. Hibernation in a tropical primate. *Nature*. 429(6994):825–26
13. Davis DE. 1976. Hibernation and circannual rhythms of food consumption in marmots and ground squirrels. *Q Rev Biol*. 51(4):477–514
14. de Jong MJ, Niamir A, Wolf M, Kitchener AC, Lecomte N, et al. 2023. Range-wide whole-genome resequencing of the brown bear reveals drivers of intraspecies divergence. *Commun Biol*. 6(1):153

15. De Napoli C, Schmidt L, Montesel M, Cussonneau L, Sanniti S, et al. 2025. Reduced ATP turnover during hibernation in relaxed skeletal muscle. *Nat Commun*. 16(1):80
16. Doherty AH, Florant GL, Donahue SW. 2014. Endocrine regulation of bone and energy metabolism in hibernating mammals. *Integr Comp Biol*. 54(3):463–83
17. Donahue SW, McGee ME, Harvey KB, Vaughan MR, Robbins CT. 2006. Hibernating bears as a model for preventing disuse osteoporosis. *J Biomech*. 39(8):1480–88
18. Dudchenko O, Batra SS, Omer AD, Nyquist SK, Hoeger M, et al. 2017. De novo assembly of the Aedes aegypti genome using Hi-C yields chromosome-length scaffolds. *Science (1979)*. 356(6333):92–95
19. Elfeky M, Tsubota A, Shimozuru M, Tsubota T, Kimura K, Okamatsu-Ogura Y. 2024. Regulation of mitochondrial metabolism by hibernating bear serum: Insights into seasonal metabolic adaptations. *Biochem Biophys Res Commun*. 736:150510
20. Evans AL, Singh NJ, Friebe A, Arnemo JM, Laske TG, et al. 2016. Drivers of hibernation in the brown bear. *Front Zool*. 13(1):7
21. Fanzani A, Conraads VM, Penna F, Martinet W. 2012. Molecular and cellular mechanisms of skeletal muscle atrophy: an update. *J Cachexia Sarcopenia Muscle*. 3:163–79
22. Fedorov VB, Goropashnaya A V, Tøien Ø, Stewart NC, Chang C, et al. 2011. Modulation of gene expression in heart and liver of hibernating black bears (Ursus americanus). *BMC Genomics*. 12(1):171
23. Fedorov VB, Goropashnaya A V, Tøien Ø, Stewart NC, Chang C, et al. 2011. Modulation of gene expression in heart and liver of hibernating black bears (Ursus americanus). *BMC Genomics*. 12:1–15
24. Fedorov VB, Goropashnaya A V, Tøien Ø, Stewart NC, Chang C, et al. 2012. Preservation of bone mass and structure in hibernating black bears (Ursus americanus) through elevated expression of anabolic genes. *Funct Integr Genomics*. 12:357–65
25. Fedorov VB, Goropashnaya A V, Tøien Ø, Stewart NC, Gracey AY, et al. 2009. Elevated expression of protein biosynthesis genes in liver and muscle of hibernating black bears (Ursus americanus). *Physiol Genomics*. 37(2):108–18
26. Fedorov VB, Goropashnaya A V, Tøien Ø, Stewart NC, Gracey AY, et al. 2009. Elevated expression of protein biosynthesis genes in liver and muscle of hibernating black bears (Ursus americanus). *Physiol Genomics*. 37(2):108–18
27. Fortes GG, Grandal-d'Anglade A, Kolbe B, Fernandes D, Meleg IN, et al. 2016. Ancient DNA reveals differences in behaviour and sociality between brown bears and extinct cave bears. *Mol Ecol*. 25(19):4907–18
28. Gehring JL, Rigano KS, Hutzenbiler BDE, Nelson OL, Robbins CT, Jansen HT. 2016. A protocol for the isolation and cultivation of brown bear (Ursus arctos) adipocytes. *Cytotechnology*. 68(5):2177–91
29. Goropashnaya A V, Tøien Ø, Ramaraj T, Sundararajan A, Schilkey FD, et al. 2021. Transcriptional changes and preservation of bone mass in hibernating black bears. *Sci Rep*. 11(1):8281
30. Gubin DG, Nelaeva AA, Uzhakova AE, Hasanova Y V, Cornelissen G, Weinert D. 2017. Disrupted circadian rhythms of body temperature, heart rate and fasting blood glucose in prediabetes and type 2 diabetes mellitus. *Chronobiol Int*. 34(8):1136–48

31. Harlow HJ, Lohuis T, Beck TDI, Iaizzo PA. 2001. Muscle strength in overwintering bears. *Nature*. 409(6823):997
32. Hellgren EC. 1998. Physiology of hibernation in bears. *Ursus*. 10:467–77
33. Herzig S, Shaw RJ. 2018. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat Rev Mol Cell Biol*. 19(2):121–35
34. Hilderbrand G V, Jenkins SG, Schwartz CC, Hanley TA, Robbins CT. 1999. Effect of seasonal differences in dietary meat intake on changes in body mass and composition in wild and captive brown bears. *Can J Zool*. 77(10):1623–30
35. Hilderbrand G V, Schwartz CC, Robbins CT, Hanley TA. 2000. Effect of hibernation and reproductive status on body mass and condition of coastal brown bears. *J Wildl Manage*, pp. 178–83
36. Hilderbrand G V, Schwartz CC, Robbins CT, Jacoby ME, Hanley TA, et al. 1999. The importance of meat, particularly salmon, to body size, population productivity, and conservation of North American brown bears. *Can J Zool*. 77(1):132–38
37. Hut R, Barnes B, Daan S. 2002. Body temperature patterns before, during, and after semi-natural hibernation in the European ground squirrel. *Journal of Comparative Physiology B*. 172:47–58
38. Jansen HT, Hutzenbiler BE, Hapner HR, McPhee ML, Carnahan AM, et al. 2021. Can offsetting the energetic cost of hibernation restore an active season phenotype in grizzly bears (Ursus arctos horribilis)? *J Exp Biol*. 224(12):
39. Jansen HT, Leise T, Stenhouse G, Pigeon K, Kasworm W, et al. 2016. The bear circadian clock doesn't 'sleep'during winter dormancy. *Front Zool*. 13(1):1–16
40. Jansen HT, Trojahn S, Saxton MW, Quackenbush CR, Hutzenbiler BDE, et al. 2019. Hibernation induces widespread transcriptional remodeling in metabolic tissues of the grizzly bear. *Commun Biol*. 2(1):1–10
41. Kahn BB, Flier JS. 2000. Obesity and insulin resistance. *J Clin Invest*. 106(4):473–81
42. Kahn SE, Hull RL, Utzschneider KM. 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 444(7121):840–46
43. Karpovich SA, Tøien Ø, Buck CL, Barnes BM. 2009. Energetics of arousal episodes in hibernating arctic ground squirrels. *Journal of Comparative Physiology B*. 179:691–700
44. Keys A, Brožek J, Henschel A, Mickelsen O, Taylor HL. 1950. The biology of human starvation.(2 vols).
45. Kim M-J, Sinam IS, Siddique Z, Jeon J-H, Lee I-K. 2023. The link between mitochondrial dysfunction and sarcopenia: an update focusing on the role of pyruvate dehydrogenase kinase 4. *Diabetes Metab J*. 47(2):153–63
46. Kirby R, Johnson HE, Alldredge MW, Pauli JN. 2019. The cascading effects of human food on hibernation and cellular aging in free-ranging black bears. *Sci Rep*. 9(1):2197
47. Kishida T, Ohashi M, Komatsu Y. 2022. Genetic diversity and population history of the Japanese black bear (Ursus thibetanus japonicus) based on the genome-wide analyses. *Ecol Res*. 37(5):647–57
48. Kisser B, Goodwin HT. 2012. Hibernation and overwinter body temperatures in free-ranging thirteen-lined ground squirrels, Ictidomys tridecemlineatus. *Am Midl Nat*. 167(2):396–409

49. Kumar S, Stecher G, Suleski M, Hedges SB. 2017. TimeTree: a resource for timelines, timetrees, and divergence times. *Mol Biol Evol*. 34(7):1812–19

50. Laidre KL, Supple MA, Born EW, Regehr E V, Wiig Ø, et al. 2022. Glacial ice supports a distinct and undocumented polar bear subpopulation persisting in late 21st-century sea-ice conditions. *Science (1979)*. 376(6599):1333–38

51. Lan T, Leppälä K, Tomlin C, Talbot SL, Sage GK, et al. 2022. Insights into bear evolution from a Pleistocene polar bear genome. *Proceedings of the National Academy of Sciences*. 119(24):e2200016119

52. Li Y, Xu W, Wang Y, Kou J, Zhang J, et al. 2022. An improved, chromosome-level genome of the giant panda (Ailuropoda melanoleuca). *Genomics*. 114(6):110501

53. Lin DC, Hershey JD, Mattoon JS, Robbins CT. 2012. Skeletal muscles of hibernating brown bears are unusually resistant to effects of denervation. *Journal of Experimental Biology*. 215(12):2081–87

54. Linke WA. 2018. Titin gene and protein functions in passive and active muscle. *Annu Rev Physiol*. 80(1):389–411

55. Liu S, Lorenzen ED, Fumagalli M, Li B, Harris K, et al. 2014. Population genomics reveal recent speciation and rapid evolutionary adaptation in polar bears. *Cell*. 157(4):785–94

56. Lotta LA, Gulati P, Day FR, Payne F, Ongen H, et al. 2017. Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance. *Nat Genet*. 49(1):17–26

57. Luu BE, Lefai E, Giroud S, Swenson JE, Chazarin B, et al. 2020. MicroRNAs facilitate skeletal muscle maintenance and metabolic suppression in hibernating brown bears. *J Cell Physiol*. 235(4):3984–93

58. Martin SL. 2008. Mammalian hibernation: a naturally reversible model for insulin resistance in man? *Diab Vasc Dis Res*. 5(2):76–81

59. McCain S, Ramsay E, Kirk C. 2013. The effects of hibernation and captivity on glucose metabolism and thyroid hormones in American black bear (Ursus americanus). *J Zoo Wildl Med*. 44(2):324–32

60. McGee ME, Maki AJ, Johnson SE, Nelson OL, Robbins CT, Donahue SW. 2008. Decreased bone turnover with balanced resorption and formation prevent cortical bone loss during disuse (hibernation) in grizzly bears (Ursus arctos horribilis). *Bone*. 42(2):396–404

61. Miller W, Schuster SC, Welch AJ, Ratan A, Bedoya-Reina OC, et al. 2012. Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change. *Proceedings of the National Academy of Sciences*. 109(36):E2382–90

62. Mugahid DA, Sengul TG, You X, Wang Y, Steil L, et al. 2019. Proteomic and Transcriptomic Changes in Hibernating Grizzly Bears Reveal Metabolic and Signaling Pathways that Protect against Muscle Atrophy. *Sci Rep*. 9(1):19976

63. Nakamura S, Yamazaki J, Matsumoto N, Inoue-Murayama M, Qi H, et al. 2023. Age estimation based on blood DNA methylation levels in brown bears. *Mol Ecol Resour*. 23(6):1211–25

64. Nasoori A, Okamatsu-Ogura Y, Shimozuru M, Sashika M, Tsubota T. 2020. Hibernating bear serum hinders osteoclastogenesis in-vitro. *PLoS One*. 15(8):e0238132

65. Nelson OL, Robbins CT. 2010. Cardiac function adaptations in hibernating grizzly bears (Ursus arctos horribilis). *Journal of Comparative Physiology B*. 180(3):465–73
66. Nelson OL, Robbins CT, Wu Y, Granzier H. 2008. Titin isoform switching is a major cardiac adaptive response in hibernating grizzly bears. *American Journal of Physiology-Heart and Circulatory Physiology*. 295(1):H366–71
67. Nelson RA, Folk Jr GE, Pfeiffer EW, Craighead JJ, Jonkel CJ, Steiger DL. 1983. Behavior, biochemistry, and hibernation in black, grizzly, and polar bears. *Bears: their biology and management*. 5:284–90
68. Nelson RA, Jones JD, Wahner HW, McGill DB, Code CF. 1975. Nitrogen metabolism in bears: urea metabolism in summer starvation and in winter sleep and role of urinary bladder in water and nitrogen conservation.
69. Nelson RA, Wahner HW, Jones JD, Ellefson RD, Zollman PE. 1973. Metabolism of bears before, during, and after winter sleep. *American Journal of Physiology-Legacy Content*. 224(2):491–96
70. Nishida K, Shimozuru M, Okamatsu-Ogura Y, Miyazaki M, Soma T, et al. 2021. Changes in liver microRNA expression and their possible regulatory role in energy metabolism-related genes in hibernating black bears. *Journal of Comparative Physiology B*. 191(2):397–409
71. Pagano AM, Durner GM, Rode KD, Atwood TC, Atkinson SN, et al. 2018. High-energy, high-fat lifestyle challenges an Arctic apex predator, the polar bear. *Science (1979)*. 359(6375):568–72
72. Pagano AM, Rode KD, Lunn NJ, McGeachy D, Atkinson SN, et al. 2024. Polar bear energetic and behavioral strategies on land with implications for surviving the ice-free period. *Nat Commun*. 15(1):947
73. Palumbo PJ, Wellik DL, Bagley NA, Nelson RA. 1980. Insulin and Glucagon Responses in the Hibernating Black Bear. *Bears: Their Biology and Management*. 5:291–96
74. Pérez-Rama M, Fernández-Mosquera D, Grandal-d'Anglade A. 2011. Effects of hibernation on the stable isotope signatures of adult and neonate cave bears. *Quaternaire*. 4:79–88
75. Perry BW, Armstrong EE, Robbins CT, Jansen HT, Kelley JL. 2022. Temporal analysis of gene expression and isoform switching in brown bears (Ursus arctos). *Integr Comp Biol*. 62(6):1802–11
76. Perry BW, McDonald A, Trojahn S, Saxton MW, Vincent EP, et al. 2023. Feeding during hibernation shifts gene expression towards active season levels in brown bears (Ursus arctos). *Physiol Genomics*
77. Perry BW, Saxton MW, Jansen HT, Quackenbush CR, Evans Hutzenbiler BD, et al. 2023. A multi-tissue gene expression dataset for hibernating brown bears. *BMC Genom Data*. 24(1):33
78. Rauch JC, Beatty DD. 1975. Comparison of regional blood distribution in Eptesicus fuscus (big brown bat) during torpor (summer), hibernation (winter), and arousal. *Can J Zool*. 53:207–14
79. Rigano KS, Gehring JL, Hutzenbiler BDE, Chen A V, Nelson OL, et al. 2017. Life in the fat lane: seasonal regulation of insulin sensitivity, food intake, and adipose biology in brown bears. *Journal of Comparative Physiology B*. 187(4):649–76

80. Rinker DC, Specian NK, Zhao S, Gibbons JG. 2019. Polar bear evolution is marked by rapid changes in gene copy number in response to dietary shift. *Proceedings of the National Academy of Sciences*. 116(27):13446–51
81. Robbins CT, Lopez-Alfaro C, Rode KD, Tøien Ø, Nelson OL. 2012. Hibernation and seasonal fasting in bears: the energetic costs and consequences for polar bears. *J Mammal*. 93(6):1493–1503
82. Rode KD, Robbins CT, Nelson L, Amstrup SC. 2015. Can polar bears use terrestrial foods to offset lost ice-based hunting opportunities? *Front Ecol Environ*. 13(3):138–45
83. Rottiers V, Näär AM. 2012. MicroRNAs in metabolism and metabolic disorders. *Nat Rev Mol Cell Biol*. 13(4):239–50
84. Rüegg MA, Glass DJ. 2011. Molecular mechanisms and treatment options for muscle wasting diseases. *Annu Rev Pharmacol Toxicol*. 51(1):373–95
85. Rutter J, Reick M, McKnight SL. 2002. Metabolism and the control of circadian rhythms. *Annu Rev Biochem*. 71(1):307–31
86. Samarel AM, Parmacek MS, Magid NM, Decker RS, Lesch M. 1987. Protein synthesis and degradation during starvation-induced cardiac atrophy in rabbits. *Circ Res*. 60(6):933–41
87. Saxton MW, Perry BW, Hutzenbiler BDE, Trojahn S, Gee A, et al. 2022. Serum plays an important role in reprogramming the seasonal transcriptional profile of brown bear adipocytes. *iScience*. 25(10):105084
88. Segawa T, Rey-Iglesia A, Lorenzen ED, Westbury M V. 2024. The origins and diversification of Holarctic brown bear populations inferred from genomes of past and present populations. *Proceedings of the Royal Society B*. 291(2015):20232411
89. Shimozuru M, Kamine A, Tsubota T. 2012. Changes in expression of hepatic genes involved in energy metabolism during hibernation in captive, adult, female Japanese black bears (Ursus thibetanus japonicus). *Comp Biochem Physiol B Biochem Mol Biol*. 163(2):254–61
90. Shimozuru M, Nagashima A, Tanaka J, Tsubota T. 2016. Seasonal changes in the expression of energy metabolism-related genes in white adipose tissue and skeletal muscle in female Japanese black bears. *Comp Biochem Physiol B Biochem Mol Biol*. 196:38–47
91. Smith TS. 2021. Polar bear reproductive and denning behavior. In *Ethology and behavioral ecology of sea otters and polar bears*, pp. 269–92. Springer
92. Srivastava A, Kumar Sarsani V, Fiddes I, Sheehan SM, Seger RL, et al. 2019. Genome assembly and gene expression in the American black bear provides new insights into the renal response to hibernation. *DNA Research*. 26(1):37–44
93. Stenvinkel P, Jani AH, Johnson RJ. 2013. Hibernating bears (Ursidae): metabolic magicians of definite interest for the nephrologist. *Kidney Int*. 83(2):207–12
94. Supple MA, Escalona M, Adkins J, Buchalski MR, Alexandre N, et al. 2024. A genome assembly of the American black bear, Ursus americanus, from California. *Journal of Heredity*. 115(5):498–506
95. Toien O, Blake J, Edgar DM, Grahn DA, Heller HC, Barnes BM. 2011. Hibernation in black bears: independence of metabolic suppression from body temperature. *Science (1979)*. 331(6019):906–9

96. Tseng E, Underwood JG, Evans Hutzenbiler BD, Trojahn S, Kingham B, et al. 2022. Long-read isoform sequencing reveals tissue-specific isoform expression between active and hibernating brown bears (Ursus arctos). *G3 Genes|Genomes|Genetics*. 12(3):
97. Vella CA, Nelson OL, Jansen HT, Robbins CT, Jensen AE, et al. 2020. Regulation of metabolism during hibernation in brown bears (Ursus arctos): involvement of cortisol, PGC-1α and AMPK in adipose tissue and skeletal muscle. *Comp Biochem Physiol A Mol Integr Physiol*. 240:110591
98. Villanueva-Canas JL, Faherty SL, Yoder AD, Alba MM. 2014. Comparative genomics of mammalian hibernators using gene networks
99. Vincent EP, Perry BW, Kelley JL, Robbins CT, Jansen HT. 2023. Circadian gene transcription plays a role in cellular metabolism in hibernating brown bears, Ursus arctos. *Journal of Comparative Physiology B*. 193(6):699–713
100. Wang G, Bonkovsky HL, de Lemos A, Burczynski FJ. 2015. Recent insights into the biological functions of liver fatty acid binding protein 1. *J Lipid Res*. 56(12):2238–47
101. Wang M-S, Murray GGR, Mann D, Groves P, Vershinina AO, et al. 2022. A polar bear paleogenome reveals extensive ancient gene flow from polar bears into brown bears. *Nat Ecol Evol*. 6(7):936–44
102. Wang RJ, Peña-Garcia Y, Bibby MG, Raveendran M, Harris RA, et al. 2022. Examining the effects of hibernation on germline mutation rates in grizzly bears. *Genome Biol Evol*. 14(10):evac148
103. Ware J V, Nelson OL, Robbins CT, Carter PA, Sarver BAJ, Jansen HT. 2013. Endocrine rhythms in the brown bear (Ursus arctos): Evidence supporting selection for decreased pineal gland size. *Physiol Rep*. 1(3):
104. Webb JG, Kiess MC, Chan-Yan CC. 1986. Malnutrition and the heart. *CMAJ: Canadian Medical Association Journal*. 135(7):753
105. Weir K, Vega N, Busa VF, Sajdak B, Kallestad L, et al. 2024. Identification of shared gene expression programs activated in multiple modes of torpor across vertebrate clades. *Sci Rep*. 14(1):24360
106. Whitney MR, Sidor CA. 2020. Evidence of torpor in the tusks of Lystrosaurus from the Early Triassic of Antarctica. *Commun Biol*. 3(1):471
107. Williams CT, Radonich M, Barnes BM, Buck CL. 2017. Seasonal loss and resumption of circadian rhythms in hibernating arctic ground squirrels. *Journal of Comparative Physiology B*. 187:693–703
108. Yan J, Barnes BM, Kohl F, Marr TG. 2008. Modulation of gene expression in hibernating arctic ground squirrels. *Physiol Genomics*. 32(2):170–81
109. Zhang H, Qi G, Wang K, Yang J, Shen Y, et al. 2023. Oxidative stress: roles in skeletal muscle atrophy. *Biochem Pharmacol*. 214:115664