CHAPTER 9

Brain Dead: The Dynamic Neuroendocrinological Adaptations During Hypometabolism in Mammalian Hibernators

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Introduction

As seasonal temperatures fluctuate, endotherms must produce body heat endogenously when ambient temperatures drop below the level of their own body temperature (T_b), in order to maintain cellular function across a range of newly established ambient temperatures. However, thermoregulatory mechanisms are fuel-expensive and require ever-increasing levels of metabolic activity, to combat the parallel increase of heat transfer to the environment by producing excess heat energy. For these reasons, animals that are exposed to extreme winter conditions must either find enough resources to maintain thermoregulatory mechanisms despite unfavorable conditions or must lower their own metabolic rate to survive without the need for massive energy stores. Furthermore, an animal's ability to forage may be restricted by daily light-dark cycles, predation-risk and food-availability, while heat-production costs and similar resource shortages may be imposed on animals suffering through food-shortage or drought, even in warm climates. By lowering T_b during times of rest an animal may preserve energy for times of greater need (e.g. during increased activity). Endothermic vertebrates that suppress energy usage and lower body temperature until conditions are more favourable for survival are considered to be in a state of "torpor," and energy expenditure can be further

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Figure 9.1 The Richardson's ground squirrel is a model hibernating animal, capable of withstanding harsh environmental conditions during the winter months by entering a state of reversible suspended animation. Source: J.M. Storey.

preserved by prolonging the time spent in torpor (model hibernator shown in Figure 9.1).

One classification scheme for heterothermic animals (i.e. those that can enter torpid states) relies on the amount of time spent in torpor. Heterothermic animals may undergo daily torpor, in which they rely on heat loss to enter a hypometabolic torpid state for less than 24 hours, and after which they typically continue foraging. Alternatively, for some animals, entering hibernation entails employing inhibitory mechanisms to lower their metabolic rate and remaining torpid for several days, while relying on internal energy stores or food caches, built up in the preceding months. Animals that hibernate may be differentiated by the 'trigger' for entering torpor; animals that enter hibernation seasonally, regardless of environmental conditions, are known as obligate hibernators, while those that enter hibernation bouts following uncharacteristically extreme seasons are known as facultative hibernators. Due to the length of time that hibernators remain hypometabolic and the phenotypic plasticity required to enter a prolonged hibernation, these animals have become characterized as the more extreme example of metabolic depression. For this reason, the following chapter will pertain mostly to examples of hibernating mammals, except when noted.

During hibernation, torpor bouts (which can last for hours to weeks) are interrupted by periodic, active euthermic phases, in which basal metabolic rate and T_b are re-adopted for one to three days (see Figure 9.2). Although the reasons for these arousal periods are debated, the process of transitioning

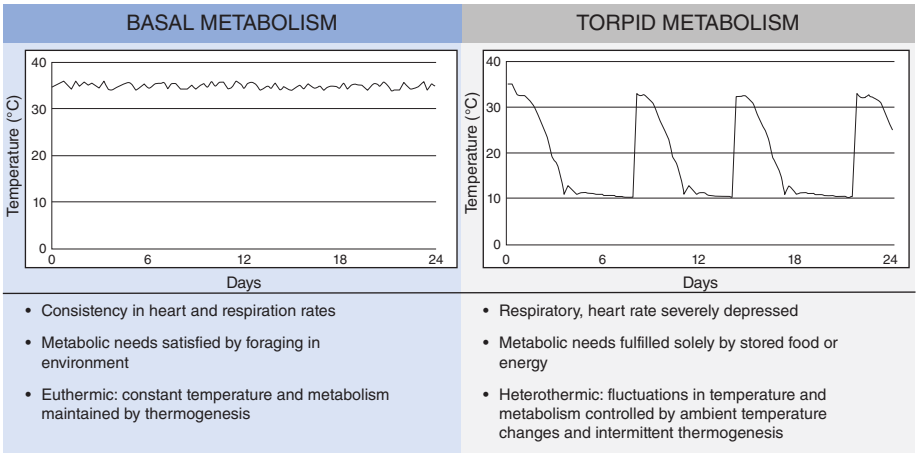

Figure 9.2 A representative comparison of body temperature and physiology of hibernators during euthermic (basal) and heterothermic (torpid) periods.

between torpor and arousal periods is characterized by extreme physiological and phenotypic changes, perhaps alluding to the necessity of arousals during hibernation. Within the central nervous system, the hypothalamus appears to control torpor-arousal transitions and seasonal rhythms, which are essential for physiological adaptations and the maintenance of energy stores. The sympathetic nervous system also mediates transitions between torpor and arousal periods, namely through the activation of shivering thermogenesis in skeletal muscle and non-shivering thermogenesis within brown adipose tissue.

The past several decades have seen physiologists, biochemists, cell biologists and neuroscientists alike come together to uncover the mysteries behind how these organisms can intricately regulate their metabolic rate, T_b, heart rate and breathing rate, as well as countless other physiological variables.

9.1 Hypothalamic regulation of hibernation

Studying the hypothalamus of hibernators is paramount for our understanding of metabolic suppression, since it is the control center for regulating body temperature (T_b) and energy homeostasis and is active over the entire temperature range of the torpor-arousal cycle, unlike the cerebral cortex (Schwartz et al., 2013). Hibernators, as opposed to pharmacologically induced hypothermic and metabolically suppressed mice or rats, provide researchers with an excellent model for studying natural metabolic rate depression and neuroprotection without introducing many experimental manipulations that could confound results.

Table 9.1 Examples of species that use obligate hibernation, facultative hibernation, or daily torpor to survive changing environmental conditions and if they use hyperphagia for fat storage or if they utilize food caches to supplement their energy stores throughout torpor.

Torpor Strategy	Order	Torpor Energy Storage	Example Genera	Confirmed Hibernating Species in Genera	Hibernating Species
Obligate Hibernation	**Rodentia**	Fat	Allactaga (Jerboa)	3	*A. euphratica, A. williamsi*
			Cynoms (Prarie dog)	3	*C. leucurus, C. gunnisoni*
			Ictidomys, Spermophilus, Urocitellus (Ground squirrel)	15	*I. tridecemlineatus, S. lateralis*
			Marmota (Marmot)	3	*M. marmota, M. monax*
			Zapus, Napaeozapus (Jumping mice)	3	*Z. hudsonius, N. insignis*
		Fat / Food	Eliomys, Muscardinus, Glis (Dormice)	3	*E. quercinus, G. glis*
		Food	Perognathus (Pocket mice)	2	*P. longimembris*
			Tamias (Chipmunks)	2	*T. amoenus, T. striatus*
	Carnivora	Fat	Ursus (Bear)	2	*U. americanus, U. arctos*
	Chiroptera	Fat	Eptesicus, Myotis, Pipistrellus (Vespertilionid bats)	6	*N. noctula, M. lucifugus*
	Eulipotyphia	Fat	Atelerix, Erinaceus (Hedgehog)	3	*A. frontalis, E. europaeus*
	Monotremata	Fat	Tachyglossus (Echidna)	1	*T. aculeatus*
	Primates	Fat	Cheirogaleus (Dwarf lemur)	2	*C. crossleyi, C. medius*
Facultative Hibernation	**Rodentia**	Fat	Cynoms (Prarie dog)	1	*C. ludovicianus*
		Food	Cricetus, Mesocricetus (Hamster)	3	*C. cricetus, M. brandti*

Table 9.1 (*continued*)

Torpor Strategy	Order	Torpor Energy Storage	Example Genera	Confirmed Hibernating Species in Genera	Hibernating Species
	Carnivora	Fat	Meles (Badger)	1	*M. meles*
	Chiroptera	Food	Lasiurus (Hairy-tailed bat)	2	*L. cinereus, L. borealis*
	Diprodontia	Fat	Cercartetus (pygmy possum)	3	*C. lepidus, C. nanus*
	Macroscelidea	Fat/Food	Elephantulus (Elephant shrew)	2	*E. edwardii, E. myurus*
Daily Torpor	**Rodentia**	Food	Peromyscus, Petromyscus (Deer mice)	7	*P. boylii, P. collinus*
			Perognathus (Pocket mice)	2	*P. californicus*
			Fukomys, Heterocephalus (Mole rat)	2	*F. damarenis, H. glaber*
	Chiroptera	Food	Macroglossus, Nyctimene, Syconycteris (Megabat)	3	*M. minimus, S. australis*
			Carollia, Glossophaga, Sturnia (Leaf-nosed bat)	3	*G. soricina, S. lilium*
	Euliptyphia	Food	Crocidurinae (White-toothed shrew)	4	*C. russula*
	Primates	Fat	Microcebus (Mouse lemur)	3	*M. murinus, M. myoxinus*

9.1.1 Hibernators as a model for studying hypothalamic-pituitary adaptations

Studying the hypothalamic-pituitary axis in hibernators is essential since it is involved in the neuroendocrine control of digestion, energy metabolism, sleep and wake cycles, circadian and circannual rhythms, immune response

and neurotransmission. The hypothalamus secretes trophic hormones into the bloodstream that stimulate receptors on the anterior pituitary, or it directly stimulates the posterior pituitary to induce hormone and neurotransmitter production and release from the pituitary. These hormones can influence hormone expression in other endocrine tissues, or affect non-endocrine tissues throughout the body.

9.1.1.1 The hypothalamic-pituitary-adrenal (HPA) axis coordinates ion homeostasis, energy metabolism and behavior in hibernators

Several lines of evidence support increased steroid synthesis in the adrenal glands during hibernation. Hibernation increases adrenal weight in several species of ground squirrel (European, Arctic, and Columbian), implicating increased adrenal activity, but molecular studies provide more information as to which processes are more active during metabolic suppression. The HPA axis involves the production and release of vasopressin and corticotropin-releasing hormone, which stimulate the production/secretion of adrenocorticotropic hormone (ACTH) in the pituitary. ACTH helps regulate ion homeostasis, glucose metabolism, reproduction, behavior and the stress response by causing the adrenal glands to produce adrenaline and noradrenaline, corticosteroids (including cortisol and aldosterone) and androgens. Advances in our understanding of the roles of these hormones in metabolic suppression will be discussed.

During hibernation, renal blood flow decreases by 90%, urine output completely stops, and creatinine builds up within the kidneys, yet organ structure and electrolyte balance are maintained, in several small hibernating mammals such as Columbian ground squirrels and jerboa (Ratigan and McKay, 2016). Control of plasma ion concentration and blood pressure during hibernation requires increased responses of the renin-angiotensinogen-aldosterone and sympathetic nervous systems to reduce cardiac output while enhancing renal vasoconstriction (Ratigan and McKay, 2016). Transmission electron microscopy of the zona glomerulus of the adrenal cortex, which secretes aldosterone, a mineralocorticoid that regulates kidney function, shows more mitochondrial cristae and a more prominent smooth endoplasmic reticulum in hibernating edible dormice (*Glis glis*) compared to euthermic and arousing dormice (Jani et al., 2013). This is consistent with reports of higher renin and aldosterone levels in hibernating organisms, and suggests an important role for the HPA axis in regulating ion homeostasis during torpor (Jani et al., 2013). Understanding the molecular mechanisms that stress-tolerant species use to reduce metabolism in kidneys could be key to prolonging the viability of organs ready for transplant in humans.

The HPA axis also regulates androgen levels to control behavior in seasonal hibernators. Western blot analysis shows that androgen receptors decrease during hibernation (Boonstra et al., 2014). Summer-active Arctic ground squirrels have more androgen receptor than Columbian ground squirrels, which

was postulated to be a species-specific protective mechanism in which Arctic ground squirrels build muscle for catabolism during hibernation so that cells are not starved. However, evidence from in-vitro tissue performance assays, muscle morphology analysis, 2D gel electrophoresis and Western blot analysis suggest that 13-lined ground squirrels prevent muscle degeneration during hibernation (Tessier and Storey, 2016). Instead, pre-hibernation accumulation of muscle mass, which is possibly regulated by seasonal variation in androgen receptor expression, is likely to be important for shivering thermogenesis during interbout arousals in Arctic ground squirrels (and less so in Columbian ground squirrels), since they are the only species of ground squirrel that can supercool their body temperature to −2.9°C. More research needs to be done to untangle the relationship between seasonally expressed hormones and hibernation as well as their roles in muscle accumulation and energy homeostasis.

9.1.1.2 The hypothalamic-pituitary-thyroid (HPT) axis may have roles in depressing metabolism during hibernation and increasing body temperature upon arousal

When low levels of triiodothyronine (T3) and thyroxine (T4) are sensed in the blood, the hypothalamus secretes thyrotropin-releasing hormone (TRH) leading to the production and secretion of thyrotropin-stimulating hormone (TSH) by the pituitary. The thyroid glands then produce T3 and T4 which downregulate processes during hibernation such as energy metabolism, heart and breathing rate, body temperature and sleep.

Total T3 and T4 levels in the plasma are regulated by different life stages (circannual rhythm) in hibernators. Specifically, T3 levels are highest during lactation and lowest during pre-hibernation hyperphagia (the period of intense eating before hibernation) in female Arctic ground squirrels (Wilsterman et al., 2015). High T3 levels are used as an indicator of elevated basal metabolic rate (metabolic rate when animals are resting but not asleep) in both laboratory and free-living animals (Wilsterman et al., 2015). Therefore, the notion that T3 levels would be lowest during the pre-hibernation period is consistent with the hibernator's need to conserve metabolic fuel for the hibernation period. An activated HPT axis leads to increased energy expenditure, which is possibly due to thyroid hormone acting as a transcription factor or its ability to increase glycogen metabolism in the liver. Increased HPT activity is observed in the hypothalamus of hibernators that are injected with TRH, which is supported by increased brown adipose tissue (BAT) and rectal temperature and increases the turnover rate of norepinephrine, despite unchanging T3 levels (Shintani et al., 2005). Cold-exposed Daurian ground squirrel BAT shows any increase in uncoupling protein 1 (UCP1) expression, which is likely involved in the mechanism of increasing BAT temperature. Furthermore deiodinases, which convert T4 to T3 before they are released from the thyroid and in peripheral tissues, are more active in several cold-exposed hibernators, including Daurian and

Richardson's ground squirrels and chipmunks, suggesting that BAT T3 availability is regulated by temperature cues (Liu et al., 2001). Thyroid hormones might increase metabolism and thermogenesis by increasing the relative levels of mitochondria although the impact of the thyroid on brown adipose tissue is still incompletely explored (Wilsterman et al., 2015).

9.1.1.3 The hypothalamic-pituitary-gonadal (HPG) axis influences torpor-arousal cycle length and seasonal reproduction

In response to the production and release of gonadotrophin-releasing hormone from the hypothalamus, the pituitary produces luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which influence the production of estrogen and testosterone in the gonads. This signaling cascade is referred to as the hypothalamic-pituitary-gonadal (HPG) axis. Hormones associated with reproductive cycles in mammals are most likely to show circannual rhythms and have served as the starting point for research into the timing involved in annual hibernation cycles.

A study measuring endogenous testosterone and dehydroepiandrosterone (DHEA, a hormone involved in regulating non-mating aggression) in Arctic ground squirrels showed that testosterone levels are highest during the mating season in the spring, and DHEA levels are highest in the late summer. Castrated ground squirrels can initiate hibernation around the same time as control squirrels but arouse from heterothermy nearly one month later than non-castrated squirrels (Richter et al., 2016). Evidence also shows that administration of testosterone following entry into torpor prevents cold-housed animals from entering deep hibernation (Lee et al., 1990). Thus, testosterone appears to have an important role in the inhibition of hibernation.

Most reproductive hormones such as prolactin and testosterone increase in abundance following arousal from torpor. An exception is that serum levels of FSH, which are high during hibernation, seem to be regulated in a seasonal fashion. Turkish hamsters exposed to a short photoperiod have inhibited HPG signaling, testicular regression, and decreased testosterone secretion, changes that are absent in animals exposed to photoperiods that simulate summer. Serum levels of FSH increase about 40 days prior to the end of hibernation, or about 100 days after exposure to short photoperiods, both in hamsters that remained euthermic in short-day cold conditions and in castrated hamsters that lack gonadal signaling to the hypothalamus (Jarjisian and Zucker, 2011). A similar study conducted on golden-mantled ground squirrels also shows increases in FSH levels within three days prior to arousal. Given that gonadal feedback to the pituitary is influential within the HPG-axis, these results suggest that seasonal stimuli influence hypothalamic signaling to the pituitary gland and ultimately, secretion of reproductive endocrine factors. It is therefore interesting that the rhythmicity of seasonal FSH secretion seems to influence the timing of arousal from hibernation and may serve as a seasonal signaling factor within the context of hibernation, possibly via secretion of melatonin

from the pineal gland. However, there may be species-specific differences in HPG regulation. FSH-secreting cells within the hibernating little brown bat brain are less active during hibernation, evidenced by less developed rough endoplasmic reticulum and Golgi-apparati, as well as smaller numbers of secretory vesicles and melatonin receptors (Azzali et al., 2003). Overall, changes in FSH and testosterone levels may provide neuroendocrine control over hibernation timing but differences between hibernator species indicate there is still much more research needed in the area of hibernator HPG signaling.

9.1.2 Hypothalamic-pineal regulation during hibernation

The control of many cyclical behaviors in mammals resides in the suprachiasmatic nucleus (SCN), a region within the hypothalamus that can integrate environmental photoperiod cues from the retina with cycles in sleep and food intake behavior. In turn, output pathways from the SCN influence endocrine (i.e. melatonin and arginine vasopressin) and neuronal signals that synchronize peripheral oscillators located in most visceral organs and in several locations within the brain, serving to maintain metabolism, appetite and activity in a circadian (i.e. daily) fashion. On the other hand, circannual rhythms correlate with seasonal changes in day length and ambient temperature and alter the release of endocrine factors that entrain (or synchronize) the SCN and the periphery to these changes. Predictably, circannual rhythm governs the onset and cessation of torpor-arousal cycles in mammalian hibernators, even those that are kept in constant environmental conditions while in captivity (Williams et al., 2014).

9.1.2.1 Melatonin may control torpor-bout length and reduce brain damage during hibernation

Through the SCN, day-length signals are encoded as endocrine signals in the form of melatonin synthesized by the pineal gland and subsequently released into the blood, most abundantly at night, during minimal photic input to the SCN. Melatonin synthesis appears to cease during hibernation in European and Turkish hamsters, ground squirrels and marmots, but is known to increase torpor bout length in ground squirrels when injected intracerebroventricularly (Yu et al., 2002). Hibernators that have undergone surgical ablation of the SCN are excellent models in which to study the effects of photoperiod input to the pineal gland during torpor-arousal cycles, although reports of their use for this purpose have so far been limited.

During arousal from hibernation melatonin levels peak. Melatonin injection forces arousal from hibernation, suggesting that it is a key player in mediating torpor bout length. Melatonin may also be involved in the neuroprotective response to increasing levels of reactive-oxygen species, which are produced as the animal warms up and breathes more rapidly (Schwartz et al., 2015). Evidence for this includes more pro-apoptotic caspase-3 expression and

less mitochondrial respiration when ground squirrels are injected with a melatonin receptor antagonist during the mid-arousal phase. Furthermore, melatonin receptor Mel1a is elevated in hibernating ground squirrel brain, heart and brown adipose tissue, as is the activity of the rate-limiting enzyme for melatonin synthesis, arylalkylamine-N-acetyltransferase (AA-NAT) (Schwartz et al., 2015; Yu et al., 2002). Thus, it is possible that Mel1a and AA-NAT are synthesized before arousal such that melatonin signaling is enhanced during the arousal phase. These studies suggest that melatonin may be important for neuroprotection upon arousal, possibly by ensuring optimal energy acquisition or by promoting antioxidant and anti-apoptotic signaling (Schwartz et al., 2015), but more research needs to be done to confirm this.

9.1.2.2 Low temperatures may directly regulate hypothalamopineal signaling in hibernators

Hypothalamic-pineal neuroendocrine signaling in hibernators may not be regulated by circadian rhythm, but instead by decreased T_b. A recent study suggests that temperature may have an important role in regulating circadian rhythms in Siberian hamsters (*Phodopus sungorus*), animals that can undergo daily torpor. Over a 48-hour period, researchers found rhythmic expression of Per1, Bmal1 and Avp proteins in the SCN and AA-NAT in the pineal gland of both euthermic and hypothermic hamsters, but protein expression was notably attenuated in hypothermic animals (Herwig et al., 2007). European hamsters exposed to a shorter photoperiod show a greater daily melatonin amplitude (higher night-time levels and lower day-time levels) as compared to hamsters exposed to a longer photoperiod, and like Siberian hamsters, this effect was exacerbated in animals exposed to cold (Revel et al., 2007). European hamsters also consistently express clock-related genes (Per1, Per2, Bmal1) in the SCN and AA-NAT in the pineal gland throughout torpor when T_b is suppressed, suggesting that circadian rhythm is lost in these animals (Revel et al., 2007). Whether this is true for obligate hibernators such as the brown bear (*Ursos arctos*), which show elevated melatonin levels at night during both the summer and the winter, is still undetermined. Day-time levels of melatonin during hibernation are higher than summer levels, suggesting that the baseline level of melatonin increases with hibernation and this elevated baseline may mask any daily changes in melatonin during the hibernation period, as opposed to these animals not having any circadian regulation of pineal signaling (Ware et al., 2013).

9.1.2.3 The signalling of seasonal changes is essential to hibernators

Obligate hibernators exhibit torpor-arousal cycles in the absence of changing temperatures and photoperiod in their underground dens, which suggests that hibernation is regulated by an endogenous 'clock' that is not under the direct control of the circadian system or its inputs. Whether this seasonal timekeeping mechanism is the result of changes in environmental conditions leading up to winter or an endogenous circannual rhythm is currently unknown, and

differences in hibernation strategy (e.g. obligate vs facultative hibernators, hibernacula suitability) are likely to have evolved unique methods of time-keeping specific to the needs of the animal. Three lines of evidence support this conclusion.

First, European ground squirrels exhibit arrhythmic T_b upon arousal from hibernation, which means that their T_b does not change with fluctuations in ambient temperature. Importantly, T_b gradually re-entrains to daily T_b rhythms, suggesting muted circadian functioning even when animals are held in constant darkness and allowed to entrain to free-running cycles (Malan, 2010). T_b arrhythmia either during or immediately following hibernation also occurs in hamsters and little brown bats. Developments in technology, including more sensitive T_b tracking devices and hibernation chambers that lower interference from daily ambient temperature fluctuations, have allowed measurement of T_b rhythms during torpor (diminished to <0.1°C). As such, circadian T_b rhythms have been shown to exist in some food-gathering species during arousals from torpor, or in captive animals measured at relatively higher ambient temperatures (~12 °C) (Malan, 2010).

Second, the SCN and its underlying circadian rhythms do not modulate the timing of hibernation bouts, as was shown by mutant hibernators displaying unaltered torpor rhythms. In an interesting study, three genotypes of Syrian hamsters with mutant endogenous circadian periods were developed in order to determine the influence of circadian rhythms on the timing of torpor-arousal cycles. Hibernation cycles of mutant and wild-type hamsters hibernating in constant darkness are not different in torpor bout length or the timing of entry into or emergence from torpor. Expecting that hamsters with shorter circadian periods would have shorter torpor bouts, the authors concluded that regulation of the temporal organization of hibernation must not involve the endogenous periods encoded within the circadian system (Malan, 2010).

Last, complete or partial ablation of the SCN was expected to disrupt the timing of torpor entry and arousal in golden-mantled ground squirrels, and experiments do show altered torpor bout duration, euthermic interval and torpor re-entry rate. Further investigation of animals with only partial SCN lesions show that although these animals display alterations in hibernation rhythms, circadian T_b rhythms are in fact maintained. It would therefore appear that changes resulting from SCN ablation are the result of a loss of SCN function, suggesting possible non-circadian roles for the SCN in hibernators (Ruby, 2003).

These studies suggested that the time spent in torpor is not affected by circadian rhythmicity, however daily T_b rhythms immediately following spring arousal have been noted in some species, raising obvious questions about the continuation and possible roles of muted circadian rhythms during hibernation. Furthermore, alternative methods of entrainment must be required for hibernating animals within their dens, due to their isolation from signals of photoperiod (daily light/dark rhythms). Given these facts, it seems possible that alternative endogenous clocks (i.e. circannual) have an influential role

in rhythmicity during torpor-arousal bouts (Malan, 2010; Ruby, 2003), in cooperation with factors signaling metabolite buildup and extreme changes in ambient temperature or surroundings that are indicative of seasonal changes in amount of daylight.

9.1.2.4 Circannual regulation of energy metabolism in seasonal hibernators

Appetite and nutrient sensing can also be impacted by temperature, photoperiod and circannual rhythm in mammalian hibernators. Time of the year affects the arousal, appetite and food-seeking behavior of different species and sexes of hibernator. Hamsters hibernate due to changes in photoperiod and arouse when they are hungry, to feed from food caches between torpor bouts. Male arctic ground squirrels also have food caches, but do not eat until after spring arousal, possibly to manufacture sperm cells before the reproductive season while still protected in their dens (Florant and Healy, 2012). By contrast, female ground squirrels and both male and female marmots arouse later in the season, when food is more readily available. Furthermore, Sciurid animals (ground squirrels, prairie dogs and marmots) given food at spring arousal in laboratory settings will not eat, suggesting that they use an endogenous mechanism to regulate appetite following arousal (Florant and Healy, 2012).

9.1.3 Hypothalamic regulation of appetite and nutrient sensing in hibernators

Hibernators are excellent models in which to study controlled overeating and insulin resistance when sampled during pre-hibernation, and also nutrient deficiency, since they can survive up to 7 months of starvation during hibernation (Wu et al., 2013). Lesion studies suggest the hibernator hypothalamus is responsible for changes in food intake and body mass. Ventral medial hypothalamus (VMH) lesions increase feeding and obesity in ground squirrels at all times of the year and lateral hypothalamus (LH) lesions decrease feeding, suggesting that these brain regions control satiety and hyperphagia, respectively. Close to the VMH and the LH, the arcuate nucleus (ARC) may be the most important area in regulating feeding response through nutrient sensing, since it is permeable to the bloodstream and allows the uptake of nutrients and hormones from the blood. The ARC expresses neuropeptide Y (NPY) and agouti-related protein (AgRP) which stimulate feeding and energy storage, which is unsurprising since most neurons in the VMH and ARC are affected by changes in fatty acid and glucose levels (Florant and Healy, 2012). At the molecular level, nutrients (glucose and free fatty acids), enzymes (AMPK and ACC) and hormones (ghrelin, insulin, and leptin) have important roles in controlling energy storage and usage in hibernators throughout the year.

9.1.3.1 Free fatty acids (FFAs) could suppress appetite at low T_b, but noradrenaline does not increase lipolysis during hibernation

Hibernators endure prolonged periods of fasting with little or no food supply, however, what controls appetite and lipolysis during hibernation is still unknown. Entry into torpor is accompanied by an increase in serum FFAs, but to date no research has shown how FFA levels change within the hibernator brain (Florant and Healy, 2012). Circulating FFAs could regulate energy metabolism and food intake by triggering nutrient sensing in the hypothalamus, as other nutrients do. FFAs, often bound by fatty-acid binding proteins (FABPs), bind to insulin receptors in adipocytes and may regulate food intake this way (Florant and Geary, 1991). Northern blot analysis shows higher FABP levels in the brown adipose tissue and skeletal muscle of hibernating little brown bats, compared to euthermic bats (Eddy and Storey, 2004). Furthermore, hibernator FABPs have amino acid substitutions that increase their binding capacity at low temperatures (near 0°C), especially compared to non-hibernators (Eddy and Storey, 2004). Increases in FABP protein levels and binding capacities during hibernation could suggest a role for FFAs in regulating energy metabolism during hibernation.

Pharmacological studies suggest interesting theories about how hibernators regulate energy metabolism at extremely low body temperature. Following injection of noradrenaline in euthermic and hibernating ground squirrels, measurements of plasma glycerol levels, used to estimate relative changes in lipolysis of white and brown adipose tissue, show that lipolysis increases only in euthermic, noradrenaline-treated animals (Dark et al., 2003). Thus, low temperatures may inhibit noradrenaline-mediated lipolysis and hibernators use alternative mechanisms to enhance fatty acid oxidation.

9.1.3.2 Ghrelin, AMP-activated protein kinase (AMPK) and acetyl-coA carboxylase (ACC)

Ghrelin is a hormone that binds to ghrelin receptors in the pituitary, activates AMPK pathways to induce feeding behavior, and increases NPY and AgRP expression in hypothalamic neurons to stimulate fatty acid synthesis (Healy et al., 2011). This is especially important in the pre-hibernation fattening period. During hibernation, plasma ghrelin levels are much lower than summer levels in grizzly bears and golden-mantled ground squirrels. Reduced but still measurable ghrelin levels may inhibit the urge to arouse and feed or may facilitate non-rapid eye movement sleep, although the latter role for ghrelin is still hotly debated (Healy et al., 2011). Major differences in ghrelin and other hormone or enzyme levels in the brain between euthermia and hibernation are summarized in Figure 9.3.

Regardless of the season, ghrelin injections into golden-mantled ground squirrels increase food intake and phosphorylation of AMP-activated protein kinase (phospho-AMPK, the active form) compared to saline-injected controls (Healy et al., 2011). AMPK is an energy-sensing enzyme that can

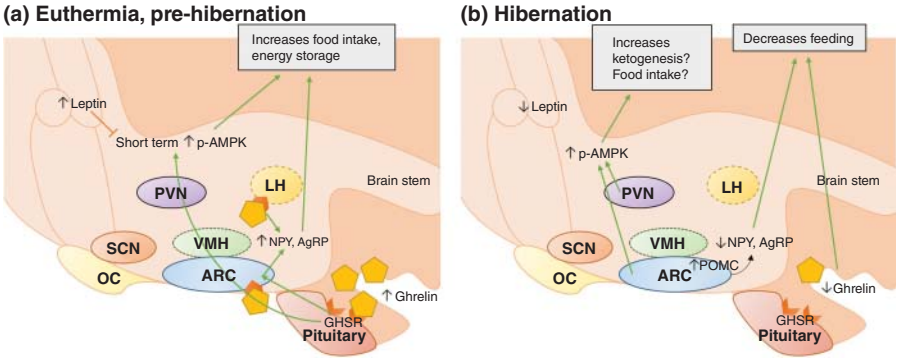

Figure 9.3 Key molecular changes that occur between pre-hibernation euthermia and hibernation that could facilitate nutrient-sensing and regulation of appetite in hibernators. (a) During euthermia, ghrelin levels are much higher than during hibernation and this influences the expression of neuropeptide Y (NPY) and agouti-related protein (AgRP), as well as phosphorylated AMP-activated protein kinase (AMPK) levels to increase food intake and energy storage during hyperphagia. High leptin levels during euthermia suppress NPY, AgRP, and AMPK activity following hyperphagia to suppress appetite. (b) During hibernation, low ghrelin levels and high proopiomelanocortin (POMC) are associated with low NPY and AgRP levels possibly to suppress appetite, but phospho-AMPK levels remain elevated, perhaps to increase fatty acid metabolism.

simultaneously increase ATP production while inhibiting energy-expensive pathways, likely by inhibiting the mechanistic target of rapamycin (mTOR), which is essential for metabolic suppression (Healy et al., 2011; Zhang et al., 2015). Yellow-bellied marmots infused with an AMPK agonist also increase food intake, emphasizing the importance of this enzyme in regulating appetite (Florant et al., 2010). In naturally hibernating ground squirrels, immunoblotting and enzyme activity assays show that the activity and phosphorylation of AMPK do not increase in brain, skeletal muscle, brown adipose tissue or liver, but increase in white adipose tissue relative to euthermic controls (Horman et al., 2005). ELISAs show AMPK to be activated in gray mouse lemur heart during daily torpor, compared to aroused animals but not in five other lemur tissues. Interestingly, acetyl-CoA carboxylase (ACC) phosphorylation (which occurs due to elevated phospho-AMPK and inhibits of fatty acid synthesis) does not increase in winter in ghrelin-injected animals, suggesting that ACC is already maximally phosphorylated during winter, to facilitate fatty acid oxidation. Together these studies suggest that AMPK may be important in food intake and nutrient sensing, thus limiting energy expenditure, but only in certain hibernator tissues (Zhang et al., 2015). AMPK activity is also regulated by insulin, leptin, glucose, FFAs and AMP/ATP ratio, which fluctuate with different times of the torpor-arousal cycle and time of year. To determine how ghrelin and AMPK influence energy metabolism in models of natural hibernation, relative total protein, phosphorylation and activity levels will need to be assessed in animals at various points of the torpor-arousal cycle and

using tissues from specific brain regions. Although unexplored in hibernators, it is possible that ghrelin regulates energy metabolism through the orexin pathway, since this peptide also plays roles in appetite, thermoregulation and regulation of the sleep-wake cycle.

9.1.3.3 Leptin, NPY and AgRP

The level of serum leptin, a hormone produced by white adipose tissue, is predictive of fat mass. To suppress appetite, leptin binds to leptin receptors in the hypothalamus, increasing proopiomelanocortin (POMC) and alpha-melanocyte stimulating hormone expression, which reduces NPY and AgRP expression in the hibernator brain (Florant and Healy, 2012). Leptin and insulin levels are elevated in serum during the pre-hibernation period, promoting hyperphagia, and decrease during torpor, when adiponectin levels increase, suppressing appetite and mobilizing fat stores (Florant and Healy, 2012).

Neuropeptides NPY and AgRP are produced by the same neurons in the ARC. Both stimulate appetite and are inhibited by leptin. NPY has been shown to reduce the anxiety associated with food foraging, resulting in more food acquisition and AgRP influences food preference. A study using qPCR shows that in greater mouse-tailed bats hypothalamic NPY mRNA levels, but not AgRP mRNA levels, increase during the pre-hibernation period, when it is essential to increase fat intake (Levin et al., 2013). Transcriptomes of 13-lined ground squirrels reveal low NPY and AgRP mRNA levels in the pre-hibernation period after hyperphagia has ceased, and high NPY and AgRP levels during post-hibernation hyperphagia. By contrast, genes involved in suppressing appetite, such as those for cocaine- and amphetamine-regulated transcript prepropeptide (CARTPT) and thyrotropin-releasing hormone (TRH), are expressed at high levels during hibernation (Schwartz et al., 2013). Although the identities of appetite-inducing and suppressing genes are still being resolved, what stimulates their expression and their mechanisms of action during hibernation have been little studied.

9.1.3.4 Adenosine as an energy-signaling molecule with roles in thermogenesis

Adenosine, an important neuromodulator in the hibernating brain, is produced within neurons when ATP reserves are low. It is postulated that the balance between intracellular and extracellular pools of adenosine controls key metabolic and neuromodulatory processes such as food intake, sleep and T_b. Specifically, extracellular adenosine may regulate torpor by binding to receptors that inhibit arousal from torpor and shivering and non-shivering thermogenesis. Extracellular accumulation could be due to inhibited adenosine kinase in astrocytes, which would prevent adenosine from being converted into AMP and would promote its passage across the plasma membrane, down a concentration gradient. In humans, brain injury that increases seizure activity is associated with low extracellular adenosine levels, probably increasing

adenosine kinase activity, which decreases intracellular adenosine levels. Alternatively, tanycytes (nutrient-sensing glial cells) may release ATP into the extracellular space to be converted into adenosine (Drew et al., 2016). Thirteen-lined ground squirrel tanycytes are more activated during torpor and early arousal, so this is a possible mechanism of adenosine build-up in the extracellular space, however, more research is required to determine the triggering stimuli for this event.

Neural accumulation of adenosine can occur following sleep deprivation. Electroencephalographic experiments confirm that hibernators are sleep-deprived and may arouse periodically to get restful sleep. Adenosine represses arousal by inhibiting cell groups involved in arousal and disinhibiting pro-sleep cell groups through adenosine receptor (AR) stimulation. Adenosine can bind to ARs from four subgroups, including A1AR and $A2_AAR$, which are responsible for dopamine and glutamate release, and $A2_BAR$ and A3AR, which regulate immune response. A1AR stimulation, which inhibits shivering and non-shivering thermogenesis, seems to be necessary for entry into torpor. Hibernating hamsters are aroused during entrance (but not from late torpor) by A1AR antagonists such as 8-cyclopentyl theophylline, while A1AR agonists such as 6N cyclohexyladenosine can induce torpor, but drugs affecting A2AR or A3AR do not alter wakefulness. Similar results are seen in obligate hibernators if A1AR is stimulated in the winter season (Drew et al., 2016). Together, these results suggest that seasonal gene expression of A1AR or temperature sensitivity to adenosine binding may be key regulatory factors driving torpor/arousal.

9.2 Hibernating species as a model for preventing neural damage

In response to an approximately 90% decrease in blood supply to the brain during torpor, hibernators decrease metabolism to match demand with supply, so as to preserve neural structure and function and retain pertinent memory. Even euthermic hibernators are more resistant to brain insults compared to non-hibernators, making studying these stress-resistant model organisms a key to better understanding of natural neuroprotection. Region-specific changes in brain size, synaptic regression and gene expression have been studied in several hibernating species. Seasonal differences in brain size have been documented for birds and mammals, in which summer brains are heavier than winter brains, showing seasonal brain plasticity (Arendt and Bullmann, 2013). Spatial learning involves synaptic signaling followed by second-messenger activation, transcription-factor translocation, gene expression and changes in neuron structure. During artificial hypothermia, comparison of hibernation-capable and non-hibernating animals attempting spatial (maze) conditioning (feeding machine or foot shock) or recognition (social or habitat) tasks, showed that

hibernation either had no effect or improved memory retention, compared to non-hibernating species, which were either unaffected or showed decreased memory retention. However, species-specific differences in memory retention following hibernation have been documented. Hibernation impairs the European ground squirrel's ability to perform spatial and operant tasks, while greater mouse-eared bats (*Myotis myotis*) could perform spatial tasks without problems following torpor (Arendt and Bullmann, 2013). These results, as well as the molecular data to be introduced, suggest that hibernating species use synaptic regression in all brain regions to preserve brain structure, but perhaps the reestablishment of these neural connections is accelerated in the regions of the brain necessary for survival immediately following arousal. For instance, the cerebral cortex, which is the first region to lose brain activity and the last to regain activity, upregulates the expression of genes involved in remodeling and plasticity, whereas in the hypothalamus, which remains active throughout hibernation, genes involved in damage response and protein turnover, feeding and satiety, seasonal timing and fuel utilization pathways are expressed (Schwartz et al., 2013).

9.2.1 The protective effects of neural regression

Hibernators must protect their brains from ischemia-reperfusion damage, cold-stress and hypoxia, among other torpor-related stressors. One way by which their brains adapt to metabolic suppression is changing the structure and biochemical composition of their neurons. Research has shown synaptic regression in animals that undergo torpor. Dendrites and axons shrink upon cold stress, decreasing the number and size of neuron branches, until arousal, when synaptic connections are re-established with no detectable loss in cognitive ability. Although this phenomenon is seen in the cortex and the thalamus of hibernating ground squirrels, the best evidence for loss of synaptic contact during torpor comes from studying the hippocampal mossy fiber terminals from the CA3 region. Tau phosphorylation increases in these neurons, suggesting a role for tau-microtubule interactions in regulating neuronal structure (Arendt et al., 2015). Transcriptomics approaches have shown more gene expression associated with cytoskeletal rearrangement in the cerebral cortex of hibernating mammals compared to the constitutively active hypothalamic region. Proteomics applied to the forebrain (encompassing the cerebellum, the thalamus, and the hypothalamus) show elevated levels of microtubule and tubulin-interacting proteins in torpid (LT) and early arousal (EA) ground squirrels. Neurofilament heavy chain (NEFH), stathmin 1 (STMN1) and dihydropyrimidinase-related protein 2 (DPYSL2) are upregulated during LT and EA but are likely deactivated during this time by post-translational modifications like phosphorylation until arousal, when synaptic regeneration by microtubule assembly is essential. Importantly, reversible phosphorylation regulates flux through metabolic pathways, and can influence neuron

structure via changes in cytoskeletal networks. Various isoforms of tubulin (TUBA1C, TUBB2A, TUBB4B) are winter-depressed (Hindle and Martin, 2013). It is possible that these proteins are insoluble, similar to aggregates of hyperphosphorylated tau or proteins such as sorcin that change conformation and increase membrane-association at low T_b, and are not detected in the soluble protein extracts used for proteomics studies. Based on their low tubulin levels and elevated levels of stathmin, which can bind tubulin dimers to remove them from the pool of soluble proteins, hibernators may form reservoirs of structural proteins that can be rapidly mobilized upon arousal to ensure synapse reformation and neuroprotection, although this mechanism of neuron plasticity during hibernation needs to be further explored (Hindle and Martin, 2013).

9.2.2 Tau phosphorylation prevents neural damage during hibernation

Tau interacts with microtubules within axons to regulate the structural stability of neurons and neuronal plasticity. Hyper-phosphorylation of tau decreases its association with microtubules, which increases the formation of neurofibrillary tangles and neuronal cell death in many models of 'taupathies', including patients with Alzheimer's or frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) (Stoothoff and Johnson, 2005). Notably, phosphorylation of tau at certain residues can decrease paired-helical formation and tau aggregation. Reversible phosphorylation of tau proteins has been documented in the brains of several hibernating species, including European ground squirrels (*Spermophilus citellus*), Arctic ground squirrels (*Spermophilus parryii*), black bears (*Ursus americanus*) and Syrian hamsters (*Mesocricetus auratus*). During torpor, both obligate and facultative hibernators show elevated tau phosphorylation that is fully reversed following arousal. In black bears, obligate hibernators that do not periodically arouse, the same trend is observed. Compared to summer and winter euthermic controls, Arctic ground squirrels were shown to have elevated tau phosphorylation at 6 of the 30 identified tau phosphorylation sites during hibernation or arousal, including S199, T205, S214, S262, S396, but phosphorylation was reversible at only half of these sites (Su et al., 2008). Interestingly, a unique conformation of tau was discovered in the cortex of hibernating black bears, suggesting a means of preventing the aggregation of hyper-phosphorylated tau. It is essential to investigate which residues are phosphorylated in various hibernating species, so as to reveal how these animals can retain neuronal integrity throughout torpor, which residues may contribute to non-aggregating phenotype and how protective mammalian torpor differs from pathological taupathies.

Phospho-tau levels increase when body temperature decreases, including during cold-stress, starvation and anaesthesia (Arendt and Bullmann, 2013). This can be explained by the altered kinetics of tau phosphatases compared

to kinases at low temperatures, resulting in more phospho-tau. One principle phosphatase, protein phosphatase 2A (PP2A), and numerous kinases, including GSK3-β, SAPK/JNK, MAP, and cdk5, are involved in regulating tau phosphorylation. Compared to summer-active Arctic ground squirrels, forebrains from torpid and aroused animals have elevated levels of inactive GSK3 β (i.e. phospho-GSK3-β (S9)), decreased levels of active phospho-GSK3-β (Y216) and less GSK3-β activity, suggesting that tau is phosphorylated before deep torpor. An environmental activity assay showed peak GSK3-β activity at 20°C, suggesting that this kinase could phosphorylate tau during entrance into torpor, when these animals are rapidly decreasing their T_b and neuronal protection is essential (Stieler et al., 2009). This interesting finding warrants a more complete time-course study on the role of GSK3β during the torpor-arousal cycle. Studies show Cdk5 is unlikely to be involved in tau phosphorylation during hibernation, because of low Cdk5 activity levels and no detection of p25, an activator of Cdk5 (Stieler et al., 2009; Su et al., 2008). Low activity of MAPKs (extracellular signal-regulated kinase, c-Jun N-terminal kinase and p38) suggests they are not involved in tau phosphorylation during deep torpor. This is seen in the brains of hibernating 13-lined ground squirrels, where phospho-p44/42 MAPK (T202/Y204) and phospho-S6K (T421/S424) levels decreased. Studying tau phosphorylation in hibernators is important because it may allow us to discern natural protective responses to stress and disease-related physiology and pathology. Similarities between patients suffering from taupathies and hibernators include synaptic reorganization, changes in neuronal connectivity and the types of neurons involved, and a decline in cognitive function, as well as hyper-phosphorylation of tau and an imbalance in the activities of tau-targeting kinases and phosphatases. In contrast to hibernating species, the brains of Alzheimer's patients show elevated activation of GSK3-β, SAPK/ JNK, cdk5, and S6K phosphorylation at T421/Y424 (Sonoda et al., 2016). Importantly, differences between model systems could indicate pathophysiological mechanisms of taupathies.

9.2.3 Glucose-oxygen deprivation and excitotoxity

Glucose-oxygen deprivation would be a real concern for hibernators if there were no molecular mechanisms in place to compensate for the approximate 90% decrease in blood perfusion to the brain during torpor. In animals that cannot adapt to glucose-oxygen deprivation (e.g. following stroke or as a result of neurodegenerative disease), decreases in cellular energy halts Na^+,K^+-ATPase activity, depolarizes cells, causes glutamate release and binding to receptors (such as N-methyl-D-aspartate (NMDA) receptors, Ca^{2+}-conducting α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors or nicotinic acetylcholine receptors), and increases calcium influx and consequent excitotoxicity, which can lead to neural cell death (Henry et al., 2007; Ross et al., 2006; Zhao et al., 2014).

Both facultative and obligate hibernators are more capable of dealing with excitotoxic stress than are non-hibernators, possibly because Na^{+}–Ca^{2+} exchangers (NCX) are more active in hibernating species, consistent with their enhanced ability to export intracellular Ca^{2+}(Zhao et al., 2014). Specifically, overexpression of ground squirrel NCX2 but not NCX1 or NCX3 in fetal rat brain cells increases non-hibernator survival following excitotoxicity (Zhao et al., 2014).

Cell depolarization leads to glutamate release and changes in glutamate levels may promote excitotoxicity tolerance in hibernator brains. A unique study, performed using *in vivo* ^{1}H-NMR spectroscopy to determine relative metabolite levels in summer-active, torpid and aroused 13-lined ground squirrel brain, showed that glutamate and glutamine levels remain low while relative levels of the inhibitory neurotransmitter GABA increase during hibernation, suggesting hibernator neuroprotection may be the result of coordinated changes in GABA and glutamate levels (Henry et al., 2007). It is possible that torpid animals do not experience the damaging effects of excitotoxicity compared to aroused hibernators and non-hibernators because glutamate is derived from glucose, which is limited in availability during this time (Drew et al., 2016). Interestingly, facultative hibernators have shorter glutamate-initiated, non-NMDAR currents than do rats, suggesting shorter periods of Ca^{2+} influx in species capable of hibernation (Zhao et al., 2014).

Released glutamate binds to receptors that control Ca^{2+} influx. Recently, it was determined that the degree of excitotoxicity depends on the amount of Ca^{2+} that enters the cell, but also the identity of the activated receptor, where influx of Ca^{2+} is more damaging if its influx is mediated by NMDARs (Zhao et al., 2014). In line with evidence showing hibernating organisms to be more tolerant of excitotoxicity, NMDAR from ground squirrel brain slices was responsible for less Ca^{2+} influx during torpor compared to interbout arousal (Ross et al., 2006). Overall, hibernators regulate ion homeostasis and avoid cell stress incurred by excitotoxicity and glucose-oxygen deprivation by increasing sodium pump activity, controlling glutamate and GABA levels, and decreasing NMDA activity.

9.2.4 Other neuroprotective molecules

Hibernation induction trigger (HIT) is a peptide that is more abundant in winter hibernators. Targeting opioid receptors with HIT or delta opioid (D-Ala 2, D-Leu 5) enkephalin (DADLE) induces hibernation (Oeltgen et al., 1982). Both HIT and DADLE increase organ viability during stress, including neuron survival. Even in non-hibernators, these molecules prove to have anti-apoptotic roles in hypoxic and ischemic brain tissue, emphasizing the importance of identifying and studying hibernation-inducible molecules for the development of novel, natural therapeutics for damaged human nervous systems (Staples et al., 2013).

9.3 Conclusions and perspectives

9.3.1 Hibernators as models for seasonal gene expression and controlled metabolic regulation

Hibernators experience downregulation of metabolism, followed by a decrease in body temperature, which can produce a further decrease in metabolism. Understanding the process of metabolic suppression, including how T_b is regulated, what the purpose of periodic arousals is, and whether circadian or circannual rhythm is essential to inducing torpor, is essential if this natural adaptation is to be applied to humans. For instance, metabolic suppression of organs for transplant could prolong their lifespan long enough for them to be transported to patients in need or stored indefinitely and properly matched to the right individual, reducing the number of organ rejections. Ultimately this knowledge could be applied to whole organisms, including astronauts needing to conserve energy until they reach their targeted destination, or to comatose patients so that their peripheral organs are not damaged from disuse. Over the decades, we have gained insight into some overarching themes of hibernator neuroendocrinology. For instance, metabolic processes coordinated by the HPA and HPT axes help to decrease metabolism and T_b during torpor and increase them upon arousal. Current theory suggests that torpor and arousal bouts are 'timed' by an additional oscillator that would allow altered endogenous periods to control timing of torpor entry and arousal, probably through control of rhythmic processes by the SCN. However, there are many aspects of hibernation that are still poorly studied. Cold exposure seems to increase the expression of deiodinases that increase T3 levels as well as UCP1 levels in many hibernators, suggesting a role of the HPT in non-shivering thermogenesis, but how thyroid hormones directly influence gene expression in hibernators is still incompletely understood. Many molecules are involved in energy-sensing and have metabolic roles in the brain, but which pathways they turn on or off is not completely understood. Preliminary research is proving promising as researchers begin to tease out which targets could be used for therapeutic hibernation, such as agonists of A1AR which can induce a hibernation-like state in non-hibernators.

9.3.2 Hibernators as models of reversible insulin resistance

Type-2 diabetics have cells that are unable to absorb glucose from the blood because they are insensitive to the hormone insulin. Although the molecular mechanisms of type-2 diabetes are commonly studied in insulin resistance-induced human cell lines and obese mouse/rat models, hibernating ground squirrels are a naturally insulin-resistant model and can be used to study the same problems. Hibernators are also excellent models for overeating and starvation without accumulating tissue damage or disease (Wu et al., 2013). As previously mentioned, ground squirrels rapidly accumulate adipocytes during hyperphagia, the intense period of eating before torpor,

which is probably facilitated by pre-hibernation insulin resistance. Insulin resistance is probably a mechanism for suppressing glucose metabolism and promoting fat oxidation during torpor, and is gradually reversed during the beginning of the hibernation period without affecting overall health. Type-2 diabetic humans have alterations in glucose metabolism, Akt signaling and peroxisome proliferator-activated receptor-c (PPAR-c)/PPAR-c coactivator 1-alpha (PGC-1a) signaling, which are all differentially regulated during hibernation in a reversible manner (Wu et al., 2013). Recent studies have shown that protein succinylation (a protein modification resulting directly from an accumulation of fumarate in tissues) increases in models of type-2 diabetic mice and diet-induced obese mice but not in ground squirrels, suggesting it to be a biomarker of mitochondrial stress in animals without the proper coping mechanisms for overeating (Thomas et al., 2012). Studying the natural neuroendocrine control of appetite and reversible insulin resistance in hibernators, perhaps by identifying post-translational modifications of key proteins other than phosphorylation, could highlight therapeutic targets in models of type-2 diabetes metabolic dysregulation to provide insights into how to treat diabetes at the molecular level going forward.

9.3.3 Hibernators as models of extreme neural stress and protection

Although some of the mechanisms that hibernators use to defend against reductions in cerebral perfusion, cold-stress and glucose-oxygen deprivation have been elucidated, how these events are triggered is still poorly understood. For instance, full-time course studies need to be made to determine which kinases and phosphatases are influencing tau hyperphosphorylation and neural regression. It is likely that core body temperature influences the expression and activities of the enzymes involved in neural regression, but more research is needed to be able to confirm this as the triggering stimulus. Alzheimer's and dementia patients suffer from memory loss as their brain deteriorates and synaptic connections are lost. Hibernators are remarkable models for the study of neural regression and the mechanisms that they use to restore synapses (and essential memory) could provide novel treatment options for those with brain disease. Hibernators also avoid brain damage by preventing ion overload in neurons. They have more active NCXs, less NMDAR-dependent influx of calcium, lower glutamine and glutamate levels and higher GABA levels during hibernation, but how these protective responses are stimulated is still unclear. Characterization of hibernator molecular mechanisms of neuroprotection could lead to clinical applications including the prevention of secondary brain damage following ischemia stroke or physical brain trauma, as well as protecting neural tissue before premeditated cerebral insults (e.g. brain surgery or a procedure that may impede blood flow throughout the body) (Frerichs, 1999).

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