Received Date : 22-Jul-2016

Revised Date : 07-Oct-2016

Accepted Date : 15-Oct-2016

Article type : Review Article

Seasonal Control of Mammalian Energy Balance: Recent advances in the understanding of daily torpor and hibernation

Martin Jastroch[1], Sylvain Giroud[2], Perry Barrett[3], Fritz Geiser[4], Gerhard Heldmaier[5], Annika Herwig[6*]

[1]Institute for Diabetes and Obesity, Helmholtz Diabetes Center & German Diabetes Center (DZD), Helmholtz Zentrum München, 85764 Neuherberg, Germany; martin.jastroch@helmholtz-muenchen.de

[2]University of Veterinary Medicine Vienna, Department of Integrative Biology and Evolution, Research Institute of Wildlife Ecology, Vienna, Savoyenstraße 1, 1160 Vienna, Austria; sylvain.giroud@vetmeduni.ac.at

[3]Rowett Institute for Nutrition and Health, University of Aberdeen, Greenburn Road Bucksburn, Aberdeen AB21 9SB, U.K.; P.Barrett@abdn.ac.uk

[4]Centre for Behavioural and Physiological Ecology, Zoology, University of New England, Armidale, New South Wales 2351, Australia; fgeiser@une.edu.au

[5]Philipps-Universität Marburg, Animal Physiology, Karl-von-Frisch-Straße 8, D-35043 Marburg; heldmaier@staff.uni-marburg.de

[6]Zoological Institute, University of Hamburg, Martin-Luther-King-Platz 3, D-20146 Hamburg, Germany

*Corresponding Author:

Dr. Annika Herwig

Zoological Institute

University of Hamburg

Martin-Luther-King-Platz 3

D-20146 Hamburg

Germany

Email: annika.herwig@uni-hamburg.de

Phone: +49 40 42838 5426

Key Words: daily torpor, hibernation, metabolic suppression, hypothalamus, brown adipose tissue

Abstract

Endothermic mammals and birds require intensive energy turnover to sustain high body temperatures and metabolic rates. To cope with energetic bottlenecks associated with the change of seasons, and to minimise energy expenditure, complex mechanisms and strategies, such as daily torpor and hibernation, are used. During torpor metabolic depression and low body temperatures save energy. However, these bouts of torpor lasting for hours to weeks are interrupted by active 'euthermic' phases with high body temperatures. These dynamic transitions require precise communication between the brain and peripheral tissues to defend rheostasis in energetics, body mass and body temperature. The hypothalamus appears to be the major control centre in the brain, coordinating energy metabolism and body temperature. The sympathetic nervous system controls body temperature by adjustments of shivering and non-shivering thermogenesis, the latter being primarily executed by brown adipose tissue. Over the last decade, comparative physiologists have put forward integrative studies on the ecophysiology, biochemistry and molecular regulation of energy balance in response to seasonal challenges, food availability and ambient temperature. Mammals coping with such environments represent excellent model organisms to study the dynamic regulation of energy

metabolism. Beyond the understanding of how animals survive in nature, these studies also uncover general mechanisms of mammalian energy homeostasis. This research will benefit efforts of translational medicine to combat emerging human metabolic disorders.

This review focuses on recent advances in the understanding of energy balance and its neuronal and endocrine control during the most extreme metabolic fluctuations in nature: daily torpor and hibernation.

Significance of torpor and hibernation

Energy budgeting is one of the most important aspects for organisms to ensure survival in seasonal environments. In mammalian and avian lineages, the evolution of sustained endothermy offered many advantages such as increased motility, brain function, growth rate and reproductive success. However, high metabolic rates require high and costly energy expenditure that must be constrained by an array of strategies to permit rheostasis and survival of extended periods of energy shortage often associated with seasonal environmental changes. Rheostasis describes the regulation of physiological processes with rhythmically changing set points, as opposed to constant steady states of homeostatically regulated systems. The coordinated down- and up-regulation of metabolism, as performed by a large number of diverse mammalian and avian species, is an especially fascinating phenomenon in this regard (1).

The physiological state of reduced metabolic rate (MR) and body temperature (T_b) in endothermic vertebrates is termed "torpor". Torpor is *the* physiological trait used for reduction of energy expenditure and permits to survive periods of energy shortage. The history of torpor research dates back to the beginning of comparative physiology uncovering extreme cases of reductions in energy use (2). In recent years, torpor has been identified in a progressively increasing species number of all mammalian subclasses (monotremes, marsupials and placentals) and avian orders, transforming a formerly taxonomically limited trait into one with broad phylogenetic roots and diversity (1, 3).

Torpor is characterized by a pronounced reduction of MR up to 98% of basal MR (4, 5). The mechanisms and pathways initializing the drop of metabolic rate are poorly understood, but entrance into torpor, which usually occurs at low ambient temperature (T_a), is generally associated with three major observations. Initially, the euthermic T_b

set point is lowered and MR reduced (6). The reduction of MR results in a fall of T_b because not enough heat is produced to maintain a high, stable T_b. The fall of T_b in turn causes a further reduction of MR. In some species, particularly in hibernators, reductions in heart and ventilation rates and inactivation of enzymes, further reduces MR to fractions of those observed during euthermia. During torpor T_b often approaches and follows T_a. Although torpid animals may thermo-conform over a wide temperature range, endothermic thermoregulation is not abandoned. When T_b reaches extremely low values torpid animals increase metabolism to defend T_b above a minimum, which varies widely among species, presumably to prevent tissue damage.

Torpor frequency, depth and duration differ widely among species ranging from prolonged periods of seasonal hibernation to short daily bouts of reduced metabolism. Hibernation is characterized by multiday torpor bouts with T_b often between 0 and 10°C but in some species from the arctic it may fall below 0°C without freezing even at a measured minimum of -2.9°C (1, 7). Importantly, bouts of metabolic depression in most species are not continuous throughout winter but multiday bouts of torpor are interrupted by periodic arousals to euthermic MR and T_b values. In contrast, daily torpor is characterized by several hours of reduced metabolic rate and T_b, usually during the rest phase of the 24 hourly (nycthemeral) activity rhythm (Figure 1). In some species like in desert-dwelling spiny mice (*Acomys* sp.), daily torpor can be extremely shallow at high T_a, where nevertheless MR decreases significantly (8). This phenotype is phylogenetically widely distributed as hibernating pygmy-possums (*Cercartetus nanus*) display similar reductions of MR at high T_a with only minor reduction in T_b (9). In between these two extremes, there is an entire scale of different torpor depth and duration including hibernation at high T_b, as found in bears or aestivation in Madagascan lemurs (10-12). However, species expressing exclusively daily torpor (i.e. daily heterotherms) differ significantly from hibernators that are physiologically capable of expressing multiday torpor with lower minimum T_b and minimum MR (1). As pointed out above, although in many heterothermic species T_b may track T_a, T_b at any stage during torpor can be controlled. The actual T_b values depend on T_a, body mass (surface-to-volume ratio) and torpor bout duration and the degree of metabolic depression. Hence, torpid animals clearly remain endotherms because they can alternate between a euthermic tachymetabolic state and a torpid bradymetabolic state

with largely reduced body functions and even can use endothermic thermoregulation while torpid (4, 5).

Cellular, biochemical and molecular adaptation during torpor

Although the biochemical changes underlying metabolic depression during torpor have not been resolved as yet, a limited number of studies suggest rather general reductions of cellular metabolism in torpid states. These studies focus on the depression of metabolic pathways (such as glycolysis), of transcription, of translation and of protein degradation (13-17). Thus, it appears that cellular metabolism is reduced in general and cell proliferation/differentiation is halted during torpor. A recent molecular survey of the genetics and proteomics during torpor showed that new genes or different proteins are not required for the expression of torpor. Instead, the phenotype of torpor may solely be related to the regulation of common metabolic pathways (18).

With reduced energy budgets, cellular homeostasis has to be maintained to counteract entropy. Thus, sufficient energy production in form of ATP is required to maintain ion homeostasis, renew proteins and decrease cellular damage. Clearly, the cellular energy consumption processes require substantial reduction and in some cases, this is caused by the reduction of temperature and enzyme activities. Mitochondria are central organelles in the conversion of nutrient to cellular energy (ATP). Temperature passively controls mitochondrial energy conversion through Q_{10} effects, but there is evidence on additional active metabolic depression that persists in isolated mitochondria from hibernating artic and thirteen-lined ground squirrels (*Spermophilus parryii)* and in torpid Djungarian hamsters (*Phodopus sungorus)* (19-22). Mitochondrial substrate oxidation is reduced during hibernation and this appears to be controlled by succinate dehydrogenase activity and possibly allosteric inhibition (19, 23, 24). The reduction in mitochondrial ATP production correlates with T_b rather than metabolic rate *per se* (21, 25).

Seasonal control of torpor

In seasonal hibernators, torpor expression is tightly linked to circannual rhythms. Hibernation is often obligatory and includes a preparation phase (pre-hibernation fattening, migration to or selection of hibernacula) with complex changes in food choice (e.g. reduction of protein intake, increased consumption and selection of specific fatty acids) and anatomy (e.g. reduction of digestive tract and reproductive organs) (26-32). The reduction of behavioural activities together with the low MR during torpor bouts reduces energy requirements during the hibernation season by up to 95% (5).

The use of daily torpor is more flexible and can either occur spontaneously (in the presence of available food), in a seasonal context; or optionally in response to energetic challenges such as food shortage or cold exposure. Daily torpor usually occurs during the rest phase of the circadian cycle and reduces daily energy requirements, as measured in captivity, by up to 60% (33). However, recent evidence from the field suggests that daily torpor may also be used to restrict foraging times to only a few hours/day in the early evening with torpor bouts lasting for most of the day and, together with passive rewarming in the sun, this reduces daily energy expenditure by up to 80% (34, 35).

In contrast to hibernation, when behaviour usually is minimised although not entirely absent, daily torpor enables maintenance of social, territorial and foraging activities of a small mammal (5, 36). Behavioural activities and ecological significance have been studied in detail and are reviewed elsewhere (37, 38). Here, we focus on the seasonal control of metabolism by the central nervous system and brown adipose tissue, as well as endocrine adjustments that occur in peripheral tissues.

Central nervous system control

Despite the general depression of cellular metabolism during torpor including silencing of cortical EEG patterns, functional brain activity persists. The specific environmental cues for the induction of torpor are still not fully understood. However, torpid animals are able to perceive and respond to external stimuli, including olfactory stimulation, precisely regulate their T_b and regularly arouse from torpor (39). Hence, the brain requires functional sites and pathways that regulate these processes as well as

protective mechanisms to ensure that tissues remain undamaged during torpid states. Changes in gene expression have been observed in the brain during deep hibernation and these show extreme regional and temporal differences providing important information about general adaptive mechanisms during torpor (40, 41). However, to date, only few studies have focused on precise anatomical information of hibernating brains to identify neuronal networks and pathways involved in the control of torpor (42, 43). The current state of knowledge suggests that the hypothalamus plays an instrumental and central role in controlling torpor similarly as in non-hibernating species.

The hypothalamus is the area of the central nervous system (CNS) that links the nervous system to the endocrine system and coordinates the majority of autonomic responses (44). This includes regulation of thermal and metabolic processes, circadian organization, sleep and reproduction and the available data suggest that various hypothalamic nuclei are involved in the control of both hibernation and daily torpor. Neurons of the preoptic area (POA), a major thermoregulatory centre, are activated during entrance into deep torpor in thirteen lined ground squirrels (*Ictidomys tridecemlineatus)* as well as pharmacologically induced torpor-like states in Djungarian hamsters (42, 45, 46). This is clear evidence for the maintenance of active thermoregulatory processes during torpor. Indeed studies in marmots (*Marmota flaviventris*) provide *in vivo* evidence that thermosensory and thermoregulatory mechanisms remain functional during hibernation, but just progressively change to a lower setpoint (47, 48).

Moreover, the suprachiasmatic nuclei (SCN) that control circadian rhythms remain active during hibernation and daily torpor, as measured by expression of the immediate early gene *c-fos* (42, 46, 49, 50). The role of the circadian clock in torpor control has been subject of many studies but its mechanisms have not been fully resolved and appear to differ substantially among species and torpor patterns (51). Spontaneous daily torpor is strongly controlled by a circadian clock and often occurs during the animal´s resting phase. In Djungarian hamsters, the molecular clockwork remains largely intact during torpor and lesion studies of the SCN provide clear evidence that the clock is involved in the timing of daily torpor bouts (49, 52, 53). Studies of deep hibernators were more controversial as they were complicated by the experimental timing during the hibernation season, thus providing evidence for, as well as against active timekeeping by the circadian system (54-63). A single study investigating molecular clock

mechanisms directly in the SCN during deep torpor in European hamsters (*Cricetus cricetus*), shows that the molecular clockwork stops, questioning its regulatory role in timing, at least through classical clockwork mechanisms (50). However, increasing *c-fos* expression in the SCN with increasing torpor bout duration in these hamsters indicates some involvement in periodic arousal mechanisms (42, 50).

Daily torpor in small mammals often occurs as an immediate response to food withdrawal, suggesting that the central nervous circuitry involved in food regulation may also play an essential role in torpor induction. The arcuate nucleus of the hypothalamus (ARC) regulates food intake and energy expenditure via anorexigenic (POMC/CART) and orexigenic (NPY/AGrP) neuronal populations. When a daily torpor like state is induced by injections of a glucose analogue that disrupts glucose oxidation (2-Deoxy-Glucose) in Djungarian hamsters, ARC neurons are activated. Hence they appear to sense the lack of fuel caused by glucoprivation (46). Moreover ARC lesions are able to prevent spontaneous daily torpor in this hamster, but torpor bouts or torpor-like bouts can be reinstated by fasting or 2-DG injections (64). Studies investigating potential ARC mechanisms regulating torpor suggest that the torpor response is mediated through NPY and NPYY1 receptor signalling (43, 65, 66). Interestingly, during deep hibernation, ARC neurons remain silent throughout the entire torpor bout in thirteen lined ground squirrels, but are activated during inter-bout arousals (42). This suggests that thirteen lined ground squirrels experience hunger during this state although they do not eat even if food is available. Whether it is an ARC signal that eventually induces the subsequent torpor bout remains unknown. Taken together the ARC appears to sense the energetic state of the animal and in turn is able to influence torpor expression. Whether this is only the case in fasting induced torpor or also in spontaneously occurring torpor remains unclear.

Microarray studies suggest that seasonal body weight changes in Djungarian hamsters or photoperiodic rats (F344) are not associated with gene expression changes observed in classical ARC systems but involve distinct mechanisms and structures (67-69). Hence, long-term seasonal body weight changes do not reflect energetic deficit. Instead of common ARC mechanisms, other structures have been identified as central players in seasonal regulation of body weight – the dorsomedial posterior arcuate nucleus and the tanycytes. Since these structures appear to be important mediators of seasonal

adaptations in energy balance, they are also interesting regions of the hypothalamus for involvement in torpor regulation.

The tanycytes adjacent to the third ventricle are a small cell population in the brain which is strongly activated during late torpor and early arousal states of hibernating thirteen lined ground squirrels (42). Tanycytes are a specific type of glial cells that bridge the ventricle to hypothalamic nuclei and are able to sense and integrate energetic state of the animal (70). This observation is particularly interesting since tanycytes have been identified as major players in seasonal changes of body mass and reproduction in birds and mammals by regulating thyroid hormone (T3) availability to the hypothalamus (71-73). In the Djungarian hamster, the seasonal reduction in tanycyte derived T3 and consequently, T3 availability to the hypothalamus is clearly critical to permit seasonal torpor (74, 75). The mechanism by which a reduction in hypothalamic T3 is permissive to torpor is unknown, but as a transcriptional regulator likely involves transcriptional changes of one or more genes. The orphan receptor *gpr50* represents one putative candidate. *Gpr50* knockout mice show enhanced propensity to fasting-induced torpor (76). *Gpr50* is highly expressed in tanycytes of long-day housed Djungarian hamsters and down-regulated upon short-day exposure (77). Whether photoperiod induced suppression of this receptor is a requirement for torpor permissiveness in Djungarian hamsters is an intriguing question. There could be a role for the temporally defined period of *gpr50* expression found from late July to early September, prior to torpor use in early winter in hamsters housed under natural photoperiod (78). Moreover, there is evidence for retinoic acid signaling in tanycytes to mediate seasonal responses in food intake and body weight through hypothalamic actions in Djungarian hamsters and photoperiodic rats (68, 79-81). Generally, RA signaling is increased under summer photoperiod i.e. the time of somatic growth and fattening and reduced under winter photoperiod. Whether RA signaling is involved in torpor regulation remains to be revealed.

Tanycytes have emerged as potential nutrient sensors in the hypothalamus (82). Consistent with tanycytes' role in nutrient sensing, thioredoxin-interacting protein (*txnip*), an important cellular metabolic regulator of cellular lipid and glucose metabolism is highly expressed in these cells (76, 83, 84). *Txnip* gene expression in tanycytes is up-regulated by fasting, further induced in fasted *gpr50* null mice and fasting-induced torpor (76). Furthermore, overexpression of *txnip* in the medial basal hypothalamus of mice facilitates features of the torpid state, i.e. reduces oxygen consumption, respiratory quotient, physical activity and brown adipose temperature (83). Consistent with a role in torpor, *txnip* is induced only in short-day torpid hamsters

(76). Taken together, current data suggest that tanycytes are likely to be involved in the preparation and the acute regulation of torpid states.

Brain control of sympathetic nervous system (SNS) activity plays a leading role in thermoregulation and may also contribute to the regulation of torpor. In non-hibernators, the raphe nuclei in the brain stem have been identified as major relay station between higher brain areas and sympathetic output, mediating vasomotor responses and controlling thermoregulatory heat production in brown fat (85, 86). Whether this pathway is identical in hibernators remains to be revealed. The importance of the SNS in torpor however, has been clearly demonstrated. Together with the antagonistic parasympathetic vagus system, the SNS regulates blood flow and metabolic rate of peripheral organs and is strongly involved in torpor initiation, torpor maintenance as well as arousal. Surprisingly, sympathetic activity is enhanced for the initiation of torpor bouts, once more emphasizing, that torpor is an active rather than a passive state (87-89).

In summary, the collection of studies on brain and torpor suggest that the hypothalamus plays a complex role in torpor regulation by integrating and regulating thermal and metabolic processes, but we are far from a complete understanding of the involvement of different nuclei and pathways involved.

Central control and peripheral action of thermoregulation

Mammalian endothermy requires sites of thermogenesis, which have to be controlled centrally to maintain body temperature. Two major forms of thermoregulatory heat production are used in mammals, shivering (uncoordinated small muscle contractions) and non-shivering thermogenesis (NST). The classical site of NST in eutherians is brown adipose tissue (BAT). BAT executes adaptive NST defending T_b in lower ambient temperatures (90). Furthermore, heat production from BAT assists in re-warming processes from torpid states (91). BAT thermogenesis is controlled by dense innervation of sympathetic nerve fibres descending from the central nervous system (92, 93). Released noradrenaline acts mainly via beta-3 adrenergic receptors inducing lipolysis and stimulation of mitochondrial oxidation rates. High energy turnover is catalysed by the mitochondrial uncoupling protein 1 (UCP1) that uncouples respiration

from ATP production by short-circuiting proton currents over the mitochondrial inner membrane. UCP1 is regulated at multiple levels: mRNA transcription is stimulated by complex hormonal (e.g. T3) and chronic sympathetic innervation.

Thermogenically competent BAT is found in eutherian mammals but not in in marsupials and birds, although there is some evidence for adaptive NST in these groups. Even in some eutherians, e.g. pigs, the UCP1 gene is inactivated resulting in poor thermoregulation. These endotherms may rely on alternative NST mechanisms, with some of those being recently discovered in UCP1-ablated mice. The physiological control of alternative heat sources in muscle and white adipose tissue is not understood as yet, requiring further confirmative studies (94, 95).

Endocrine seasonal control

An increasing body of evidence sheds light on the endocrine signalling of metabolic depression. In most hibernators pronounced changes in physiological parameters like body mass, reproductive axis or thermal insulation precede the torpor season. These are caused by, or are associated with altered hormonal states, which are prerequisites for seasonal torpor. For example, the role of melatonin in seasonality is well-documented. Melatonin secretion from the pineal gland precisely reflects night length and is used by seasonal species to time seasonal events including torpor expression (96, 97). The melatonin feeds back to the brain via the pars tuberalis of the pituitary gland that possesses a large number of melatonin receptors in seasonal and non-seasonal species (98). Decreased duration of melatonin release in summer permits TSHß release from the pars tuberalis, which in turn increases the expression of type II deiodinase (*Dio2*) in the tanycytes. *Dio2* encodes for the enzyme converting thyroxine (T4) to the bioactive triiodothyronine (T3) that is a crucial driver of seasonal adaptations in birds and mammals (71-73). Although changing day length and light period alter the melatonin and downstream signal, this consequently leads to very diverse physiological adaptations in various species. There is also an array of peripherally secreted hormones that feedback to the brain on the metabolic status of the body. Most of these hormones induce behavioural and physiological changes in the brain such as appetite and thermoregulation. Many hibernators such as bats, marmots, ground squirrels or bears fatten in autumn in order to survive on fat stores over the winter (27). The enlarged fat

stores release elevated leptin concentrations that peak at the beginning of the hibernation period. Furthermore, leptin resistance is thought to be developed during pre-hibernation to counteract its anorexigenic and catabolic effects, thus allowing small hibernators to store fat at maximal amount considering their body size (99-101). Interestingly, injection of leptin, indicating large fat stores, reduces depth and duration of daily torpor (102). In contrast, the gut-produced orexigenic hormone ghrelin concentration gradually increases during summer to reach high values at the autumnal-hyperphagic period (27). Peripheral injections of ghrelin cause the increase in food intake at all seasons, even in aphagic hibernators at the start of hibernation (103). In particular in the grey mouse lemur, it was reported that ghrelin promotes fat accumulation after periods of chronic food deprivation in winter. Plasma levels of ghrelin were positively associated with body mass gain during re-feeding (104). Conversely, levels of peptide YY, an anorexigenic hormone that belongs to the PP-fold family (promoting fat use), were negatively correlated with body mass gain in the same individuals in winter. Ghrelin was also implicated in thermoregulation as peripheral ghrelin injection results in deeper (lower T_b) and more robust torpor bouts of mice, *Mus musculus* (105). This effect could be abolished by ablation of the ARC (arcuate nucleus). Interestingly, the anorexigenic hormone glucagon-like peptide 1 (GLP-1) associates positively with torpor depth in food-restricted mouse lemurs (*Microcebus murinus*) in summer but not in winter (104). However, regulation of torpor by GLP-1 does not involve NPY neurons since intra-cerebro-ventricular injections of GLP-1 in the ARC did not alter NPY mRNA levels, contrasting observations in fasted mice (106).

In contrast to the usually extensive fattening in hibernators for winter survival, small mammals such as the Djungarian hamster, shrews and voles use the opposite strategy by losing body mass before winter season, thus saving on absolute energy expenditure (107, 108). Reduction of fat mass leads to low levels of circulating leptin, which may be permissive for daily torpor expression, at least in dunnarts (*Sminthopsis macroura*) and Djungarian hamsters (102, 109).

Reduced body mass in winter also comprises the reduction of lean mass and the inhibition of growth. Interestingly, pharmacological inhibition of the growth axis strongly impacts on torpor behaviour in Djungarian hamsters (110). A recent study with long acting somatostatin receptor agonists administered intraperitoneally indicates a potential role for somatostatin in the induction of torpor. Pasireotide, a somatostatin receptor subtype 5 (SSTR5) agonist, increased the propensity to torpor in short-day acclimated Djungarian hamsters from 2-3 bouts per week

to almost a daily occurrence. Furthermore, pasireotide prolonged the duration of torpor, which in some hamsters occasionally extended into the dark phase of the circadian cycle when under natural conditions torpor would have ceased (110). However, octreotide a SSTR2 agonist was almost ineffectual. As pasireotide does not cross the blood-brain barrier, pasireotide must act at a peripheral site to enhance entry into torpor. The additional effect of pasireotide to reduce growth would favor the pituitary as the site of action and a possible neuroendocrine involvement, but an indirect action via other peripheral sites or a direct effect of the agonist on BAT cannot be dismissed (111). The ability of pasireotide to increase the propensity of torpor may point to a role of somatostatin in short-day induced torpor. Although causality has not been established to date, somatostatin is the only neuropeptide found to be increased in short-day exposed Djungarian hamster in a largely distinct population of neurons in the arcuate nucleus (112).

Decreases of gonadotropins and gonadal hormones inhibit reproduction during winter in many hibernators. Testosterone can effectively block daily torpor or hibernation in some but not all species, whereas lack of testosterone does not induce daily torpor (113, 114). Also prolactin can inhibit daily torpor to some extent (115). On the other extreme, the reproductive system of hibernating bears appears to be in an entirely different state as the females give birth and suckle their offspring during the hibernation season (12). Moreover, it appears that many small marsupials and bats use torpor to permit reproduction on limited resources. In some of these species testosterone does not inhibit torpor effectively (116).

The diversity of seasonal strategies in different heterothermic species hampers attempts to draw general conclusions on hormonal control of torpor. The hormonal status and the interpretation of hormonal signals may differ substantially depending on the ecological and physiological context of each species.

Seasonal control by nutrition and dietary lipids

Dietary lipids have profound impact on daily torpor and hibernation. Both experimental trials and field studies discovered that increased polyunsaturated fatty acids (PUFA) content in the diet and in white adipose tissue reserves associates with torpor bout duration and lowers minimal T_b in torpor, thus promoting energy savings (117-121). Linoleic acid (LA) belonging to the n-6 family was often the major dietary PUFA

provided. However, feeding n-6 PUFA-enriched diets did not enhance torpor in all species (122) and interestingly, diets enriched with n-3 PUFA, namely linolenic acid, seem to inhibit torpor expression (123, 124).

Enhanced torpor expression mediated by dietary PUFA was linked to the rise of n-6 PUFA content and the concomitant reduction of saturated fatty acids (SFA) in lipid reserves as well as in phospholipid (PL) membranes of almost all body tissues (125, 126). The differential distribution of lipid types associated with various expression of torpor was also observed independently of dietary manipulation or selection (127-130), suggesting selective uptake of lipids by the gut or selective utilization of lipid types during torpor. Indeed, the grey mouse lemur in winter increases selectively the oxidation of SFA, retaining n-6 PUFA (*i.e.* LA) in body tissues and membranes, while increasing torpor expression in response to food restriction (131). Such change in lipid composition is expected to ensure proper body functions at low T_b during torpor. The specific molecular mechanisms by which PUFA affect torpor, are to date entirely unknown, but it is possibly not simply a reflection of lipid fluidity.

A potential mechanistic role of PUFA in modulating torpor could be linked to the maintenance of the cardiac function at low T_b (132). Supporting evidence arises from a recent study in Syrian hamsters (*Mesocricetus auratus*) during hibernation, showing specific roles of n-6 and n-3 PUFA in the regulation of the cardiac sarcoplasmic reticulum (SR) calcium ATPase (SERCA), a key enzyme ensuring proper calcium handling and hence heart function (133). Cardiac SERCA activity was positively associated with LA content and negatively with the amount of docosahexanoic acid (DHA, 22:6 n-3) in cardiac SR PL of torpid hamsters (133). Moreover, very high amounts of DHA in the SR PL were found in normothermic summer individuals or those that failed to hibernate in winter, and in long-day acclimated deer mice (*Peromyscus maniculatus*), that were reluctant to enter torpor, DHA in muscle tissue was increased by 2-fold ((124, 129, 133). Further, it seems that altered SERCA activities determined the minimum T_b reached by Syrian hamsters during hibernation (133). Modulation of SERCA activity may also occur during daily torpor, since faster calcium reuptake rates into the SR were found in daily heterotherms (134). Future work will have to address many unknowns concerning n-6 PUFA in maintaining body rheostasis during daily torpor and hibernation, in particular controlling cardiac function.

Conclusions

Seasonal control of energy balance and torpor are amongst the most fascinating physiological adaptations in endotherms. Although being a widely used strategy described in many mammalian orders, relatively little is known about the mechanisms driving and controlling metabolic depression. Variation in the physiological context and characteristics of torpid states substantially between species complicate the identification of common pathways. Recent advances in the understanding of CNS control over seasonal adaptation in general, fostered by technological progress in analytical methods enabling large scale sensitive and comparative approaches, assists to dissect the interplay between environmental and internal factors controlling torpor. Translating knowledge from torpor research will benefit many clinical settings that attempt to manipulate metabolism.

Acknowledgements

We thank Julian Mercer for the invitation to submit this review that originated during the symposium "Seasonal control of mammalian energy balance" at the 9th International Congress of Comparative Physiology and Biochemistry hosted by the University of Krakow in August 2015. Additional funding was provided by the British Society for Neuroendocrinology.

Funding

This work was supported in part by BBSRC grant (BB/M001504/1) to PB, DFG Emmy-Noether HE6383 to AH, German Center for Diabetes Research (DZD) to MJ.

Duality of interest

The authors declare that there is no duality of interest associated with this manuscript.

Contribution Statement

All authors drafted and critically revised this manuscript.

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Figure Legends

Fig. 1. Torpor episodes in daily torpor and during hibernation. T_b body temperature, MR metabolic rate. A: Spontaneous daily torpor in short day acclimated Djungarian Hamster, *Phodopus sungorus,* at 5 °C ambient temperature and food *ad libitum*. B: torpor bout during hibernation in a Common Dormouse, *GLis glis*. In *Glis* metabolic rate was recorded with high resolution to visualize intermittent ventilation in the torpid state.