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Torpor induction in mammals: recent discoveries fueling new ideas

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When faced with a harsh climate or inadequate food, some mammals enter a state of suspended animation known as torpor. A major goal of torpor research is to determine mechanisms that integrate environmental cues, gene expression and metabolism to produce periods of torpor lasting from hours to weeks. Recent discoveries spanning the Metazoa suggest that sirtuins, the mammalian circadian clock, fibroblast growth factor 21 (FGF21) and lipids are involved in torpor induction. For example, sirtuins link cellular energy status to the mammalian circadian clock, oxidative stress and metabolic fuel selection. In this review, we discuss how these recent discoveries form a new hypothesis linking changes in the physical environment with changes in the expression of genes that regulate torpor induction.

Torpor in mammals

All animals require energy from food to sustain normal physiology and behavior. The quantity and quality of food provided by the environment might fluctuate from day to day or season to season, thus natural selection has favored animals adapted to these fluctuations. Torpor, the controlled lowering of metabolic rate, body temperature and physical activity, is a highly successful adaptation that various mammals use to cope with periods of low food availability (Figure 1, Box 1).

As homeotherms, mammals maintain a constant warm body temperature (approximately 35–38°C) despite continuous temperature variation in their environment. The ability to maintain a constant body temperature has contributed to the evolutionary success of mammals, allowing the group to invade unoccupied niches and become distributed worldwide. Some mammals periodically turn down their internal thermostat and enter torpor as a means to survive periods of low food availability and then rewarm and return to a normal level of activity when the environment becomes favorable. Various degrees of torpor are observed over a phylogenetically wide range of mammals (Figure 2, Box 1), from the shallow torpor seen in starved laboratory mice (*Mus musculus*) [1] to daily torpor in Siberian hamsters (*Phodopus sungorus*) [2] to the champion of all torpid mammals, the hibernating Arctic ground squirrel (*Spermophilus parryii*), which exhibits body temperatures as low as −2.9°C [3] (Box 1).

The expression of torpor is responsive to the availability of food. It is well established that the laboratory mouse can enter daily torpor when fasted [1]. Variable expression of torpor in response to the availability of food was elegantly demonstrated in the Eastern chipmunk (*Tamias striatus*) [4,5]. Chipmunks are food-storing hibernators that hoard food within their burrow before the onset of torpor [6]. During the hibernation season, chipmunks whose food hoards were supplemented above the level available by normal foraging had a higher frequency of normothermy, with minimum body temperatures 5–10°C higher than that of chipmunks whose food stores were not supplemented [5].

Many natural compounds have been shown to induce a torpor-like phenotype in mammals (reviewed in [7]). However, the mechanisms that integrate the environmental, physiological, metabolic and molecular changes associated with torpor are largely unknown. Interestingly, much has been learned from genetic mutations in both invertebrate and vertebrate organisms (Box 1) that phenocopy the biochemistry and physiology of mammalian torpor. In this review, we highlight recent discoveries that demonstrate how nutrient-sensing molecules integrate gene expression with cellular energy status and the increasingly recognized role of lipids as signaling molecules. We also discuss potential mechanisms of mammalian torpor induction that are beginning to emerge based on these new discoveries.

Timing of torpor

Discovery of the link between environmental changes, metabolism and cellular time-keeping has great potential for determining the mechanism(s) that induces torpor. Recent studies in mice demonstrate a connection between cellular nutrient status and the core molecular machinery of the mammalian circadian clock [8,9]. Both hibernation and daily torpor are often preceded by a period of fasting or reduced food consumption that alters cellular nutrient status [10]. Nutrient status is indicated by the ratios of intracellular [5′-AMP] to [ATP] and [NAD$^+$] to [NADH] [11,12]. Fasting increases both ratios thereby reducing cellular nutrient status. The animal can respond to lowered nutrient status by remodeling metabolism so that stored lipid becomes the primary source of fuel [13].

Circadian oscillation of cellular nutrient status and metabolic substrate use has been observed in mammals and is tightly coupled to feeding schedule [8,11,14]. When the timing of food availability is altered, the metabolic cycle is shifted to correlate with the new feeding schedule [15–17]. Substantial evidence supports the existence of a clock mechanism that paces circadian oscillations in response to regularity of feeding (reviewed in [18]). This suggests that availability of food, in addition to other environmental factors such as day length and temperature, might strongly regulate the timing of torpor induction.

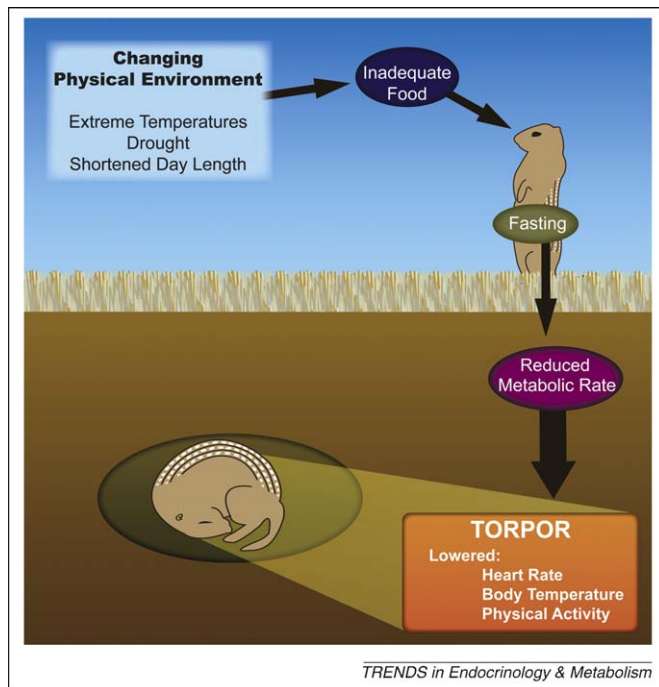*Corresponding author:* Andrews, M.T. (mandrews@d.umn.edu).

Figure 1. Environmental influence on the induction of torpor in mammals. Torpor has evolved as an adaptation for conserving energy during periods of inadequate food. Many regions of the earth are subject to extreme fluctuations in day length, temperature and rainfall. These environmental changes directly affect the quantity and quality of food available to resident animals. When an animal's food becomes scarce, it is forced to enter a period of fasting. Some animals conserve energy during periods of low food availability by reducing their metabolic rate and entering torpor. In mammals, torpor conserves fuel reserves by drastically reducing body functions that depend on the continuous input of energy. The most recognizable characteristics of torpor in mammals are profound reductions in heart rate, body temperature and physical activity.

The expression of various genes that encode metabolic enzymes follow a circadian pattern in the brain and liver of mouse [19]. A key mitochondrial enzyme involved in shutting down carbohydrate oxidation during the deep torpor associated with mammalian hibernation, pyruvate dehydrogenase kinase isoenzyme 4 (PDK4; [20,21]), also shows a circadian pattern of expression in rat (*Rattus norvegicus*) cardiomyocytes [22,23] and in mouse liver, aorta and kidney [19]. Genetic mutations that disrupt function of the core circadian clock proteins [circadian locomotor output cycles kaput (CLOCK) and brain and muscle aryl hydrocarbon receptor nuclear translocator (BMAL1)], are associated with metabolic disorders in mice and humans (reviewed in [24]). Such a synergistic relationship between the mammalian circadian clock and metabolism is probably required to integrate an animal's changing environment with molecular and metabolic cycles to induce torpor (Figure 1). An integrative mechanism that accounts for this synergy has been suggested for mammals [25], but its experimental demonstration has only just begun.

Nampt and SIRT1 link cellular nutrient status to the circadian clock and torpor

Recent studies by Ramsey *et al*. [8] and Nakahata *et al*. [9] reveal a direct link between metabolism and the core transcription-translation feedback loop of the mammalian circadian clock in mice. At the center of this link are nicotinamide phosphoribosyltransferase (Nampt), the rate limiting enzyme in the major mammalian NAD$^+$ salvage pathway [26,27] and silent mating type information regulation 2 – homolog 1 (SIRT1), an NAD$^+$-activated deacetylase [28]. These studies demonstrated that cellular nutrient status, reflected by intracellular [NAD$^+$], has a strong influence on transcription of genes at the core of circadian rhythms and metabolism (reviewed in [24,29–31]). These discoveries suggest a new and exciting avenue of investigation that is likely to reveal mechanisms that integrate nutrient status, biological timing and metabolic substrate use during torpor induction. In fact, the concentration of NAD$^+$ in liver of the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) entering torpor is on average 2–3 times higher than that found in summer-active ground squirrels, or in ground squirrels that are about to exit torpor and return to activity [32], suggesting that Nampt and SIRT1 are likely to be activated during torpor induction.

A hypothetical role for Nampt, SIRT1 and the core circadian clock genes in the induction of torpor can be easily envisioned (Figure 3). First, as a result of reduced food supply due to a changing environment, an animal enters a period of fasting. During a fast, cellular [NAD$^+$] increases as a result of nutrient depletion [16,17,19,22], causing SIRT1 deacetylase activity to increase. SIRT1 activation of Nampt activity upregulates the NAD$^+$ salvage pathway, maintaining elevated [NAD$^+$] and reinforcing SIRT1 activity. Activated SIRT1 also deacetylates the circadian clock protein BMAL1, inactivating the heterodimeric transcription activation complex that BMAL1 forms with CLOCK. Inactivation of the BMAL1:CLOCK complex inhibits expression of the circadian clock genes *Per* and *Cry* [33,34], disrupting the normal circadian feedback loop and causing the clock to either slow or stand still. This would prolong a transcription profile typical of an animal's inactive phase (e.g. during sleep) for genes regulated by the circadian clock.

The molecular details of gene regulation by the mammalian circadian clock have been reviewed recently [24]. In mice, genes encoding key rate-limiting enzymes of glycolysis, lipolysis and cholesterol metabolism are regulated by the circadian clock in liver [19] and there is evidence that lipid metabolism in adipocytes is regulated by BMAL1 [35]. Additionally, SIRT1 activation of lipolytic pathways would shift metabolism away from carbohydrates and toward catabolism of lipids [36,37]. The combined effects of prolonged fasting, SIRT1 activity, inactive/night-time mode circadian clock and a switch to lipolysis would ultimately poise the animal for entry into torpor (Figure 3).

The nuclear receptors REV-ERBα, a negative regulator of BMAL1:CLOCK; and RORα, a positive regulator of BMAL1:CLOCK, might also play a role in regulating mammalian torpor [38–41]. Supporting the model in Figure 3, the expression of REV-ERBα in the heart of Siberian hamsters, an animal that exhibits daily torpor, was higher in torpid than in normothermic animals [42]. Carey and coworkers also observed that the liver concentration of cholesterol sulfate, a ligand of RORα [43], was low during torpor and rose slightly during interbout arousals (IBAs; brief, periodic arousals from hibernation torpor) and in summer-active animals [44]. Activation of

Box 1. Torpor: Definitions, types and forms

Torpor has been defined as a temporary physiological state characterized by a controlled lowering of metabolic rate, body temperature (T_b) and physical activity below that considered normal [80]. Lyman defined the induction of torpor operationally as a process that begins with lowering the metabolic rate followed by body cooling and inactivity [81]. This operational definition makes a distinction between torpor and hypothermia - a state that begins with body cooling. Although the whole animal phenotype is the easiest way to recognize torpor, there might be great variation in the magnitude of body temperature reduction and duration of torpor bouts in different species (Tables 1 and 2) [82].

The definition of torpor has been extended to the molecular level. During torpor, metabolism switches from consuming carbohydrates to consuming lipids. This switch can be observed using respirometry to measure and calculate the ratio of exhaled CO_2 to inhaled O_2, called the respiratory quotient (RQ). The RQ of several torpid animals is close to 0.7, indicating that lipid is the major source of fuel.

Discovery-based approaches have been used to define the torpor transcriptome [83–86], proteome [69,87,88] and metabolome [32,44,89], with emphasis on hibernating mammals. In combination, these surveys show the commonality and complexity of the torpor phenotype and have fueled ideas for research into the molecular mechanisms that induce and maintain torpor.

Table 1. Types of torpor in mammals[a]

Torpor	Characteristics	Example species				
	T_b range; torpor length range	Group	Common name	*Genus species*	T_b range, °C	Torpor length range
Shallow or daily torpor	10 °C ≤ T_b ≤ 25 °C; torpid ≤ 1 day	Marsupials	Honey possum	*Tarsipes rostratus*	5–24.5	1.5–19.5 hours
		Insectivores	White-toothed shrew	*Crocidura suaveolens*	17.9–21.6	3–8 hours
		Carnivores	Striped skunk	*Mephitus mephitus*	28–28.4	9–22 hours
		Rodents	Siberian hamster	*Phodopus sungorus*	12.3–22	4.5–20 hours
Deep torpor or hibernation	T_b < 10 °C; torpid > 1 day	Marsupials	Pygmy possum	*Cercartetus nanus*	1.3–5.9	6–23 days
		Insectivores	European hedgehog	*Erinaceus europaeus*	5.4–15	5–10 days
		Bats	Little brown bat	*Myotis lucifugus*	1.3–9	10–40 days
		Carnivores	American black bear[b]	*Ursus americana*	28.4–32.3	45 days
		Rodents	Arctic ground squirrel	*Spermophilus parryii*	(−2.9)–7.5	5–33 days
		Primates	Fat-tailed lemur[c]	*Cheirogaleus medius*	9.3–30	7–30 days
Estivation[d]	T_b < 30 °C; torpid days to weeks	Rodents	Edible dormouse	*Glis glis*[e]	25	> 21 hours

[a]Summarized from Geiser and Ruf [82] unless noted.
[b]Among carnivores, the bears are classified by Geiser and Ruf [82] as hibernators based on length of the torpid period.
[c]Dausmann *et al.* [90] showed that the fat-tailed dwarf lemur's body temperature is uncontrolled and therefore fluctuates with that of its environment.
[d]Estivation is similar to hibernation but occurs in response to desiccation stress in hot climates.
[e]Wilz and Heldmaier [91].

Table 2. Types of torpor in invertebrates and reptiles

Torpor type	Description	Example organisms	Reference
Diapause	Developmental arrest in response to unfavorable environmental conditions resulting in low availability of food	Nematodes (*Caenohrabditis elegans*)	[92]
		Insects (*Drosophila* and *Lymantria*)	[93]
		Rotifers (*Brachionus plicatilus*)	[93]
		Earthworms (*Lumbricus terrestris*)	[93]
		Crustaceans (*Artemia franciscana*)	[93]
		Terrestrial gastropods (*Helix spp.*)	[93]
Estivation	Metabolic arrest in response to high temperatures and low availability of water	African lungfish (*Prototerus spp.*)	[94]
		Salamanders (*Siren lacertina*)	[95]
		Land snails (*Otala lactea*)	[96]
		Edible dormice (*Glis glis*)	[91]
Brumation (reptiles only)	Period of lowered metabolism and reduced physical activity in response to cold temperature and reduced food availability	Cottonmouth snake (*Agkistrodon piscivorous leucostoma*)	[97]
		Western fence lizard (*Sceloporus occidentalis*)	[98]
Anhydrobiosis	An extremely dehydrated state in which an animal shows no signs of metabolism but can quickly return to normal metabolic activity in the presence of water	African midges (*Polypedilum vanderplanki*)	[99]
Anaerobiosis	Reduction of metabolic activity in response to hypoxic environments	*Daphnia*	[100]
		Drosophila	
		C. elegans	

BMAL1:CLOCK by RORα might be necessary for IBAs to occur. These results suggest that REV-ERBα and RORα are components of a mechanism that integrates cellular nutrient status, the circadian clock and metabolism (reviewed in [41]). Further investigation of these nuclear receptors in active and torpid animals will possibly reveal more about the regulation and maintenance of torpor.

SIRT1 activity might also blunt potential oxidative stress associated with torpor by direct activation of hypoxia-inducible factor (HIF)-2α and the resulting
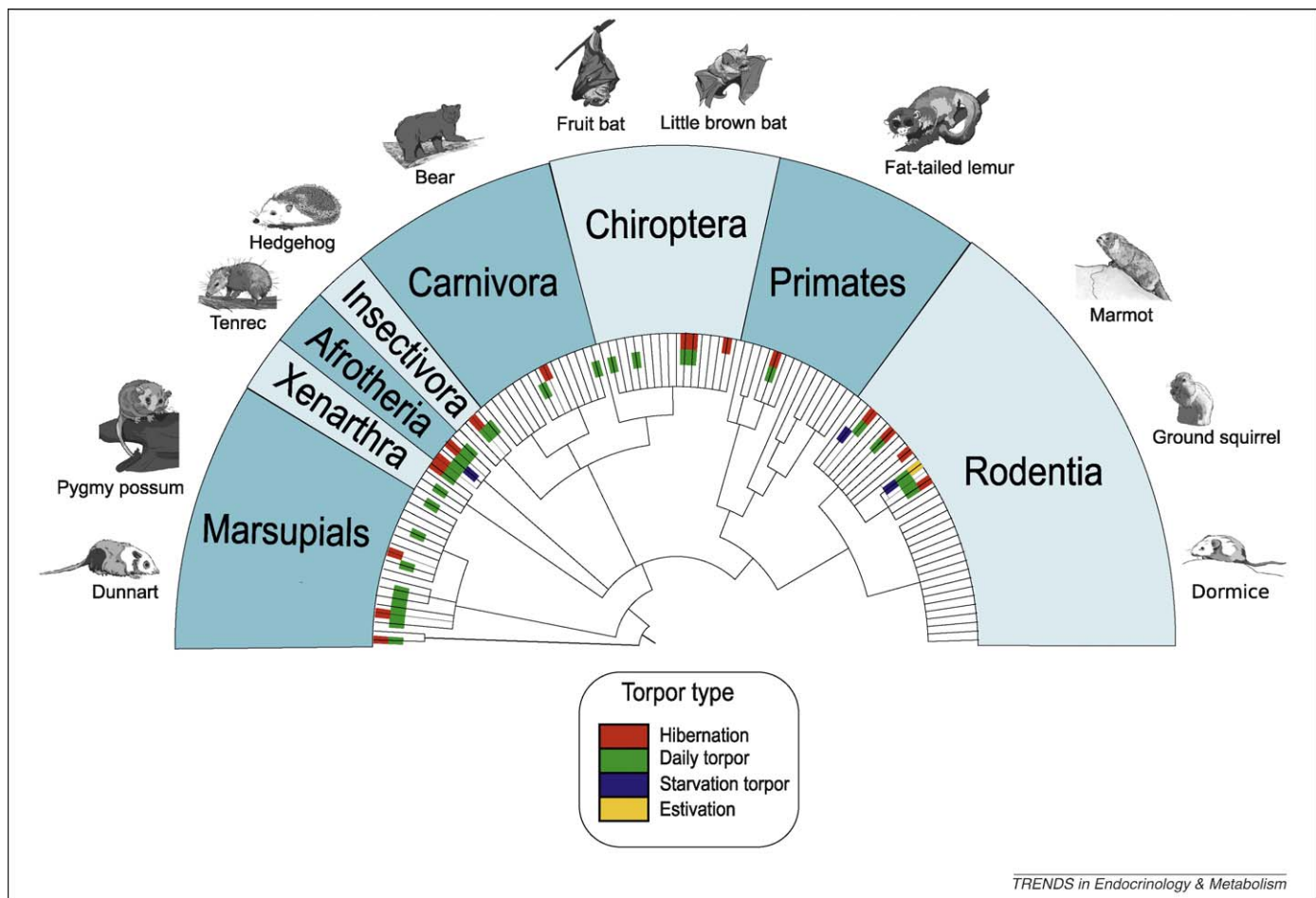
Figure 2. Mammalian groups that display torpor. Torpor is widespread among mammals, suggesting that the relevant metabolic pathways and physiological processes are ancestrally derived. The dendrogram shows the phylogenetic relationships of families (each leaf of the dendrogram) within eight mammalian groups in which some members exhibit torpor. The type(s) of torpor exhibited is indicated by color on the leaves of the dendrogram (see figure key). Representative animals that exhibit a form of torpor are depicted for each group except the Xenarthra (anteaters and armadillos). Groups of land-dwelling mammals for which torpor has not been described in any member are excluded from the dendrogram. Excluded groups include the superorder Cetartiodactyla (cattle, camels and deer) and the orders Proboscidae (elephants), Dermoptera (flying lemurs), Lagomorpha (hares and rabbits), Scandentia (tree shrews), Perissodactyla (horses and rhinoceroses) and Pholidota (pangolins). The dendrogram was prepared using the Interactive Tree Of Life (iTOL) [103].

expression of HIF-2α target genes [45] (Figure 3). It is not currently understood how animals resist cellular damage due to reduced oxygen utilization during torpor, or the rapid reperfusion of tissues during arousal. A recent study of Arctic ground squirrels found no evidence of oxidative stress in brain cortex or liver tissues of torpid or cold-adapted squirrels [46]. In the same study, brown adipose tissue showed limited oxidative stress in association with arousal from torpor. It has been hypothesized that anti-oxidant defense mechanisms are set in place before torpor induction [46]. SIRT1 activation of hypoxia-inducible factors might provide a mechanism for preparation of these defenses.

FGF21 induces lipolysis in response to long-term fasting and promotes torpor

In mouse hepatocytes, SIRT1 interacts directly with peroxisome proliferator activated receptor (PPAR)α to activate genes involved in lipolysis [47]. Overexpression of SIRT1 activates PPARα targets including the gene encoding fibroblast growth factor 21 (FGF21) [47]. FGF21 is a hormone that promotes lipolysis and predisposes 24-hour fasted mice toward entry into torpor [48–50]. In mice, torpor is a natural

response to starvation conditions [1] and circulating FGF21 levels rise after a 12-hour fast [48,49]. FGF21 levels rise in humans after 7 days of fasting [51].

Promotion of torpor including activation of lipolysis, reduction of serum glucose and increase in serum ketones by overexpression of FGF21 in mice resembles the torpor phenotype of natural hibernators. Interestingly, liver mRNA levels for pancreatic triacylglycerol lipase (*Ptl*), PTL-related protein 2 and the PTL cofactor colipase were increased. These same lipolytic genes were also induced in wild-type mice when PPARα agonists were administered [49]. PTL is a common gastrointestinal enzyme that is expressed in non-pancreatic locations such as heart and white adipose tissue in hibernating thirteen-lined ground squirrels [20,52]. We have shown that both human and ground squirrel PTL hydrolyze triacylglycerols at temperatures as low as 0°C [53]. Lipolysis at low body temperatures is important for the survival of mammals that rely on stored lipids throughout the hibernation season.

It was recently shown that FGF21 overexpression in the mouse liver stimulates transcription of peroxisome proliferator-activated receptor gamma coactivator-1-α (PGC1-α) [54]. Induction of PGC1-α results in regulation of essential
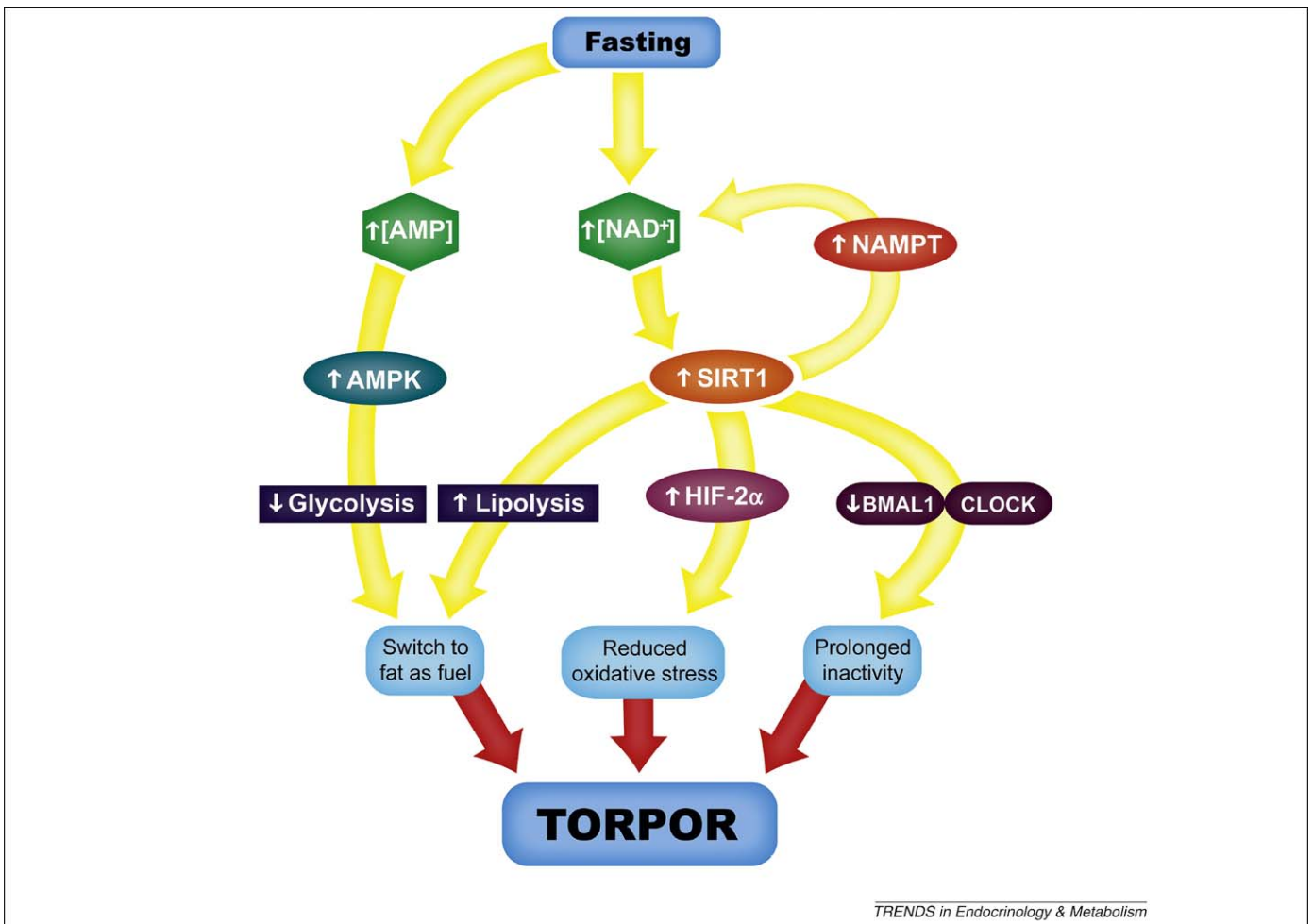
Figure 3. A model for torpor induction in mammals that links reduced availability of food with the energy status of the cell, the mammalian circadian clock and response to oxidative stress. An animal begins to fast as a result of reduced availability of food in the environment. Fasting results in depletion of cellular nutrients and increased intracellular concentrations of AMP and NAD$^+$. As intracellular [NAD$^+$] increases, the activity of SIRT1 deacetylase is also increased. Activated SIRT1 can enter the nucleus and deacetylate many targets including the core circadian clock protein, BMAL1. Deacetylation of BMAL1 inhibits transcriptional activation of genes that are regulated by the heterodimeric transcription factor complex formed between BMAL1 and CLOCK (BMAL1:CLOCK). This prolongs a gene expression profile that is typical of an animal's inactive period. SIRT1 activates nuclear receptor proteins that promote transcription of enzymes that function in lipolysis and in resistance to hypoxic stress (HIF-2α). Activation of lipolytic enzymes is part of the switch from carbohydrate to lipid-based metabolism. SIRT1 increases biosynthesis of its own activating cofactor, NAD$^+$, by deacetylation and activation of NAMPT. Fasting induced nutrient depletion also causes increased intracellular [AMP] and results in increased activation of AMPK. AMPK acts to promote the switch to lipid-based metabolism by down-regulating glycolysis. Small molecules are indicated by green hexagons. Proteins are indicated by ovals of various colors. Biochemical processes are indicated in purple boxes. Whole organism phenotypes are indicated in light-blue boxes. ↑, increase; ↓, decrease.

genes for lipolysis, the tricarboxylic acid cycle, oxidative phosphorylation and gluconeogenesis. Fasted *Fgf21* knockout mice have reduced PGC1-α expression and impaired lipolysis [54]. However, the ability of *Fgf21* knockout mice to enter torpor has not been reported. The transcriptional activity of PGC1-α is modulated by SIRT1 (reviewed in [55]). These results in mice suggest a mechanism of torpor induction that connects SIRT1, PPARs and FGF21, which deserves further investigation.

Adenosine monophosphate-activated protein kinase (AMPK)
Proteins that sense and respond to cellular nutrient status are also likely to play an important role in the induction of torpor. Such sensing mechanisms ensure that preparedness for entry into torpor is coordinated with environmental conditions. Adenosine monophosphate-activated protein kinase (AMPK) is highly sensitive to cellular nutri-

ent status in terms of the [5′-AMP] to [ATP] ratio (Figure 3; reviewed in [56]). AMPK is activated when cellular [5′-AMP] rises and is antagonized by increased cellular [ATP]. Indeed, raising the level of 5′-AMP by intraperitoneal injection induces hypothermia in mice [57], although the relevance of increasing extracellular [5′-AMP] to the activity of AMPK is unclear [58].

The traditional role of AMPK in the cell is to maintain a low [5′-AMP] to [ATP] balance by downregulating pathways that consume ATP and upregulating those that produce ATP. In rat hepatocytes, AMPK conserves carbohydrates by inhibiting glycolysis under conditions that mimic the fasting-induced increase of cellular [5′-AMP] [59] (Figure 3). Kawaguchi and coworkers [59] showed that AMPK downregulates transcription of the key glycolytic enzyme, L-pyruvate kinase, through phosphorylation and inactivation of the transcription factor, carbohydrate responsive element binding protein (ChREBP) [60]. AMPK

Box 2. Ceramides

In mammals, ceramides function in cell type differentiation, response to stress, aging, apoptosis, and arrest of the cell cycle [77]. The function of ceramides in torpor is not completely understood; however, they might contribute by reducing stress of reduced oxygen consumption and low temperature by altering the local physical properties of cell membranes and/or influencing cell signaling across the membrane [101]. The ceramide molecule consists of a single long-chain base (sphingosine, dihydrosphingosine, or 4-hydroxysphinganine) to which a fatty acid is attached (Figure I). In mammals the most abundant ceramides contain fatty acid chains of 16–24 carbons [101].

Enzymatic pathways that respond to multiple cellular stresses determine the ceramide composition of a cell and thus, ceramides might represent a point of integration for the stress response. Because ceramides play a central role in the metabolism of sphingolipids and cell signaling, they have been called ''biostats'' or monitors of cellular stress status [101]. Recent studies suggest that ceramides might be central to linking excess dietary saturated fatty acids, and the inflammatory response of cytokines, to metabolic disorders including obesity, diabetes and metabolic syndrome [102].
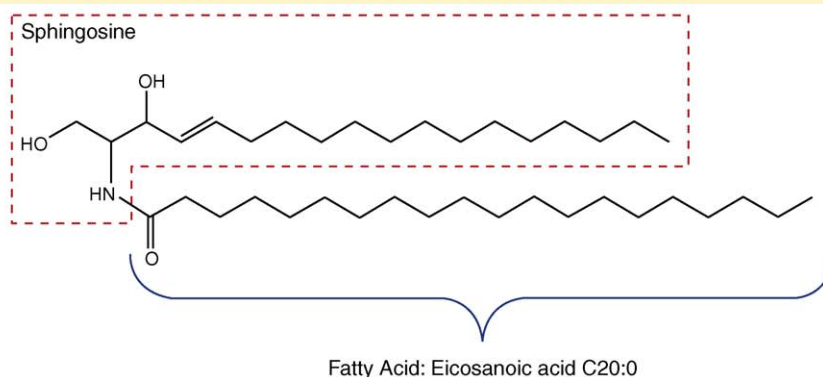
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Figure I. The chemical structure of a C-20 ceramide. The ceramide molecule depicted consists of a single long-chain base, sphingosine (boxed), to which a 20-carbon, saturated fatty acid (eicosanoic acid, C20:0) is attached (indicated by a bracket in the diagram). In *C. elegans*, the synthesis of ceramide species having C20–C22 fatty acid chains was found to be required for optimal survival under hypoxic conditions [76]. In mammals, the fatty acid chain attached to sphingosine can range from 16 to 24 carbons in length.

also influences energy homeostasis at the level of the whole organism and might be regulated by leptin [61,62], adiponectin [63] and ghrelin [64], this last being a known inducer of hypothermia and torpor in starved mice. Taken together, these studies suggest that 5'-AMP plays a broad role in torpor induction.

AMPK might play an important role in regulating the flow of metabolic fuel during torpor. Many hibernating mammals switch to lipid catabolism and survive solely on their own fat stores for up to 6 months [3]. It is therefore important that enough fat is stored as white adipose tissue and that fat stores are not depleted prior to spring arousal and the resumption of feeding. Similar to hibernating mammals, survival of the metabolically suspended dauer larvae of the nematode *Caenorhabditis elegans* depends on frugal use of lipid reserves. Larvae carrying a null mutation in the alpha 2 subunit of AMPK are unable to inactivate adipose triacylglycerol lipase (ATGL), an enzyme that catalyzes the initial step of triacylglycerol mobilization in mammals [65], and therefore they rapidly burn through their lipid stores, resulting in death before exiting dauer stage [66]. It is not known if this mechanism is conserved in mammals.

Lipids and torpor
The switch from carbohydrate to lipid as fuel is one of the most striking changes observed during torpor. This switch is essential for animals that enter long-term torpor such as hibernating mammals (reviewed in [67]) and those that estivate (a state of torpor induced by high temperatures or

dehydration) (Box 1). In many hibernating species, the animal will not ingest food for several months and must strictly conserve fuel reserves. In Siberian hamsters, the switch to lipid catabolism is also seen during daily torpor, where glucose is catabolized for the first few hours followed by a gradual switch to lipids as torpor continues [2]. We have shown in hibernating ground squirrels that the heart and brain preferentially use lipid-derived ketones as fuel [68]. At low body temperatures, glucose and the ketone D-β-hydroxybutyrate (BHB) are both transported across the blood–brain barrier, but BHB is used preferentially, highlighting the strict conservation of carbohydrates [68]. In the heart, both substrates are catabolized, but only carbon atoms derived from BHB enter the tricarboxylic acid (TCA) cycle [68]. This is due to increased expression of PDK4 in the heart which blocks conversion of the glycolytic product pyruvate to acetyl-CoA; whereas catabolism of BHB results in two molecules of acetyl-CoA that directly enter the TCA cycle [21,68,69]. Among the substances capable of inducing hypothermia and a torpor-like phenotype, the thyroid hormone derivative 3-iodothyronamine [70] also promotes lipolysis in Siberian hamsters [71]. Lipid catabolism was increased for several hours following administration of 3-iodothyronamine [71] suggesting that this naturally occurring hormone has an important role in torpor maintenance.

White adipose tissue also functions as an endocrine organ (reviewed in [72,73]). Lipid mobilized from white adipose tissue might signal when lipid stores are sufficient for entry into torpor and/or initiate the switch from carbo-

hydrate to lipid-based catabolism. For example, we hypoth-esized that a seasonal increase in circulating lipids liber-ated from white adipose tissue, combined with a decrease in insulin signaling, is responsible for inducing PDK4 expression in hibernators [21]. This hypothesis is sup-ported by a study showing that synthetic agonists and long-chain fatty acids activate PPARα and increase PDK4 expression in heart and skeletal muscle [74].

Ceramides

As stated earlier, the mechanisms that protect mammals from oxidative damage during repeated cycles of torpor and arousal are unknown. The ceramides, a group of sphingolipid derivatives (Box 2), might have an important role in this protection (reviewed in [75]). A study in *C. elegans* demonstrated that specific ceramide species con-taining fatty acid chains of 20–22 carbons (C20–C22 cer-amides) protect against exposure to anoxia [76]. Menuz and colleagues identified a mutation in the homolog of yeast longevity assurance gene 2 (*Hyl-2*) that lowers sur-vival during anoxia [76]. The investigators determined that tolerance of anoxia required functional *Hyl-2*, which encodes a ceramide synthase. The Hyl-2 ceramide synthase preferentially synthesized C20–C22 ceramides. A second ceramide synthase, Hyl-1, preferentially pro-duced C24–C26 ceramides and was unable to confer anoxia tolerance.

The specificity of the hypoxia-resistant phenotype to a specific set of ceramides suggests that the C20–C22 cera-mides function in a hypoxia-protective pathway. Mammals have six isoforms of the ceramide synthetic enzyme, dihy-droceramide synthase (CerS), which produce C16–C24 cer-amides. All mammalian CerS enzymes produce multiple ceramide species, with CerS1 and CerS2 producing C20 ceramides and CerS2, CerS3 and CerS4 producing C22 ceramides [77]. In a study of ceramide species composition in growing, hypoxic mouse hearts, Noureddine and cow-orkers observed a significant increase in concentration of dihydro-*N*-palmitoyl-D-erythro-sphingosine (DHC-16-Cer, the precursor of C16 ceramide) and a significant decrease in C20 ceramide concentration in the right ventricle [78]. These changes in ceramide composition occurred in associ-ation with adaptation of the right ventricle to hypoxia, and suggest that shorter chain ceramides might participate in the cellular response to low oxygen stress.

The mechanistic role of ceramides in mammalian torpor is currently unknown. Recent studies show that levels of the ceramide breakdown product sphingosine are reduced in the liver of torpid compared with active thirteen-lined ground squirrels [44]. In addition, sphingomyelins, of which ceramides are a component, are reduced in the cerebral cortex of hibernating Yakutian ground squirrels (*Spermophilus undulatus*) [79]. It is currently unclear if these results indicate a preference for specific, protective ceramides or a general economy of lipid molecules in these hibernating species.

Future directions and medical applications

Management of food resources is a crucial concern across the Metazoa, and the mechanisms involved are likely to be highly conserved. These same mechanisms might play a

> ### Box 3. Unanswered questions
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> This review identifies several molecules that are potentially central to understanding the mechanism of torpor induction. We have presented a hypothetical model that integrates these molecules and connects environmental changes to the induction of torpor (Figure 3). The roles that these molecules play in torpor are yet to be established. Research questions that need to be addressed include:
>
> 1. What is the role of sirtuins in torpor induction? We discussed multiple potential roles for sirtuins in torpor including influence on the circadian clock, alteration of metabolism and inducing hypoxic stress response.
> 2. What is the role of FGF21 in the induction and maintenance of torpor? The activation of lipolysis is important for maintenance of long-term torpor. The role of FGF21 in naturally-induced torpor and its relationship to sirtuins in torpor induction should be explored.
> 3. How do lipid molecules function in torpor? Lipids play a central role as a fuel source, but their putative role as signaling molecules in torpor is not fully understood.

role in conserving energy by inducing torpor. In this review, we have emphasized the potential roles of recently discovered, highly conserved mechanisms of managing cellular energy status including SIRT1, the circadian clock, FGF21 and lipid molecules. The recent discovery of these mechanisms and their striking ability to reproduce many of the phenotypic changes observed in naturally induced torpor, present a new context for investigating some of the major questions of torpor research (Box 3). The roles of Nampt, SIRT1, FGF21, ceramides and other lipid mol-ecules have yet to be thoroughly studied in mammals that naturally enter torpor. Doing so will probably reveal how environmental cues are interpreted and translated and how the metabolic fuel switch is achieved. Understanding these mechanisms is also likely to accelerate the develop-ment of pharmaceutical interventions for obesity, diabetes and the metabolic syndrome.

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