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THEME AND VARIATIONS: HETEROTHERMY IN MAMMALS

Organ Protective Mechanisms Common to Extremes of Physiology: A Window through Hibernation Biology

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Synopsis Supply and demand relationships govern survival of animals in the wild and are also key determinants of clinical outcomes in critically ill patients. Most animals' survival strategies focus on the supply side of the equation by pursuing territory and resources, but hibernators are able to anticipate declining availability of nutrients by reducing their energetic needs through the seasonal use of torpor, a reversible state of suppressed metabolic demand and decreased body temperature. Similarly, in clinical medicine the majority of therapeutic interventions to care for critically ill or trauma patients remain focused on elevating physiologic supply above critical thresholds by increasing the main determinants of delivery of oxygen to the tissues (cardiac output, perfusion pressure, hemoglobin concentrations, and oxygen saturation), as well as increasing nutritional support, maintaining euthermia, and other general supportive measures. Techniques, such as induced hypothermia and preconditioning, aimed at diminishing a patient's physiologic requirements as a short-term strategy to match reduced supply and to stabilize their condition, are few and underutilized in clinical settings. Consequently, comparative approaches to understand the mechanistic adaptations that suppress metabolic demand and alter metabolic use of fuel as well as the application of concepts gleaned from studies of hibernation, to the care of critically ill and injured patients could create novel opportunities to improve outcomes in intensive care and perioperative medicine.

Supply-demand balance is critical to survival

Hibernation is an evolved strategy that conserves energy seasonally and confers a significant survival advantage to individuals among select but diverse mammalian lineages when faced with environmental austerity or uncertainty. Animals exposed to extreme environmental conditions that challenge the abilities of their metabolic systems to produce energy are forced to choose one of two options: either procure more resources or require fewer of them. While many mammals have achieved biological success by consuming more food, defending larger territories, hoarding, or migrating, others have adapted to energy stress by using torpor to decrease metabolic utilization of fuel, thereby surviving for extended periods only on endogenous or stored energy. Torpor can occur daily or, in hibernation, extend over days to weeks through a highly regulated and systemic reduction in metabolic heat production and thus a lower need for energy and nutrients. As a byproduct of these responses, the animal temporarily converts from a homeothermic to a heterothermic pattern of body temperature.

Similar to this evolved adaptive strategy that matches physiological demand to limited resources and energy-stress (Staples and Buck 2009), a fundamental principle in the care of critically ill and perioperative patients is the maintenance of a careful balance between metabolic supply and demand. Heart failure, sepsis, severe trauma and hemorrhagic shock, ischemia-reperfusion injury, and preservation of organs for transplant are common clinical examples in which the supply of oxygen and metabolic fuels to tissues is unable to meet energetic demands. Critically ill patients are dependent on a narrow supply-demand margin for survival, and currently available medical techniques aim, for the most part, to expand this margin by increasing supply

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rather than by lowering demand. The current medical armamentarium is replete with drugs and techniques designed to increase blood pressure, cardiac output, and delivery of oxygen and substrate, in an effort to restore homeostasis at basal, or often elevated, metabolic rates characteristic of acute critical illness; yet very few are aimed at inducing a hypometabolic state as a strategy for reinstating temporary equilibrium. This emphasis on restoration reflects in part the classical explanation of metabolic regulatory mechanisms only in terms of supply while placing little emphasis on demand (Hofmeyr and Cornish-Bowden 2000; Oliver 2002). There are some exceptions described below.

Long-term, definitive therapeutic solutions clearly require a careful tuning and restoration of supply to match normal demand in order to facilitate healing processes. However, in the setting of acute injury, cardiovascular failure, and stroke, or as critical illness progresses, manipulating the body's ability to reduce metabolic demand represents an attractive strategy for expanding the window of protection and for providing the time required for recovery of organ function.

Natural adaptations to reduce metabolic rate

Depression of metabolic rate as a primary conservation strategy during torpor is known to combine specific changes in baseline physiology (reduced heart and respiratory rates, digestion, renal function, and the movement of muscles) with global biochemical adaptations (selective suppression of non-critical cellular functions), but remains incompletely understood at a cellular and molecular level (Storey and Storey 2004). Proposed critical regulatory control mechanisms that are involved in lowering the rate of ATP turnover during torpor include: (1) inhibition of protein synthesis and changes in the assembly of ribosomes (Frerichs et al. 1998; van Breukelen and Martin 2001); (2) post-translational modifications of proteins via phosphorylation (Brooks and Storey 1992), SUMOylation (Lee et al. 2007), and, more recently, acetylation (Hindle et al. 2014); (3) reduced activity of ion-motive ATPases and the flux of ions across membranes (MacDonald and Storey 1999; Malysheva et al. 2001); (4) mitochondrial proton-leak (Staples and Brown 2008); (5) increased sensitivity to adenosine receptor agonists (Jinka et al. 2011); and (6) deregulation of circadian clock proteins (Revel et al. 2007; Malan 2010; Williams et al. 2012).

Under conditions when metabolic supply is acutely or chronically reduced to critical levels, non-hibernating mammals can also invoke protective phenotypes that share some characteristics with torpor. Although responses to reduced supply of blood and oxygen have been studied in many mammalian tissues, intrinsic cardioprotective phenomena have been most extensively characterized. Following brief, acute sublethal ischemic episodes, the heart protects itself through a complex *ischemic preconditioning* (IPC) response, which prevents cardiac cell-death during subsequent prolonged ischemia (Murry et al. 1986). Chronic exposure to hypoxia or repetitive acute exposures to sublethal ischemia can result in an energy-conserving phenotype of reversible and reduced cardiac contractility known as *myocardial hibernation* (Camici et al. 2008), perhaps an unfortunate medical use of the term that forever foils comparative physiologists doing literature searches on ''hibernation''.

IPC is observed across numerous species, including chickens (Liang and Gross 1999), rats (Li and Kloner 1993), mice (Sumeray and Yellon 1998), rabbits (Cohen et al. 1991), dogs (Murry et al. 1986), pigs (Schott et al. 1990), sheep (Burns et al. 1995), and humans. This intrinsic tolerance involves an early or ''classic'' preconditioning lasting 1–4 h (Sack et al. 1993), followed by a delayed ''second window of protection'' that offers less intense protection for a prolonged period of 12–72 h (Baxter et al. 1997). The efficacy of IPC has elegantly invalidated a previous paradigm of energy deficiency as the main mechanism leading to cell-death during ischemia-reperfusion. Rather, preconditioning modifies the consequences of reperfusion by preserving the survival of cells and affording protection against post-ischemic contractile dysfunction and ventricular arrhythmias. Like torpor, IPC is clearly an integrated, whole-organism protective response, as evidenced by the fact that rendering single tissues ischemic results in remote conditioning and in subsequent protection of organs not directly affected by the initial ischemia (Thielmann et al. 2013). Remote IPC occurs via an as-yet-unknown neurohumoral mechanism, at least partially mediated by circulating opioids (Patel et al. 2002). Several endogenous and exogenous agonists of G-protein-coupled receptors trigger IPC, thereby sharing some signaling pathways also implicated in torpor. Opioids (Liang and Gross 1999), norepinephrine (Banerjee et al. 1993), and bradykinin (Ebrahim et al. 2001) signal through the δ-opioid, the α_1-adrenergic, and the β_2-adrenergic

receptors, respectively, all activating components of the reperfusion injury salvage kinase (RISK) pathway and eventually converging on mitochondrial targets such as K_{ATP} channels and the permeability transition pore (Cohen et al. 2000). Anoxia-tolerant species like the fresh-water turtle also employ signaling through δ-opioid receptors (Pamenter and Buck 2008) and through downstream activation of pro-survival kinases such as the extracellular signal-regulated kinase (Greenway and Storey 2000). Adenosine signaling offers another intriguing link between IPC and torpor. While multiple studies have identified its crucial roles in myocardial adaptation to ischemia, recently involving the circadian rhythm protein *Per2* (Eckle et al. 2012), within the CNS adenosine has also been linked to induction of torpor in mice (Staples and Buck 2009; Iliff and Swoap 2012) and of hibernation in ground squirrels (Jinka et al. 2011). Similarly, 5′-adenosine monophosphate (5′-AMP)-activated protein kinase, a well-known sensor and regulator of cellular energy status, is implicated in IPC leading to cardioprotection (Young 2008), while at the same time administration of 5′-AMP induces a deep and reversible hypometabolic state in non-hibernating mammals (Zhang et al. 2006; Daniels et al. 2010). In contrast to torpor however, the metabolic phenotype of 5′-AMP-induced hypometabolism is characterized by minimal alterations in lipid metabolism, with regulation of carbohydrate metabolites playing important roles both during the hypometabolic state and during the following recovery process (Zhao et al. 2014).

IPC can protect tissue from further ischemia-reperfusion injury, but it is when myocardial tissue receives chronically reduced coronary blood flow that it enters the well-recognized state of impaired resting contractile function and reduced energy consumption of the heart known as myocardial hibernation (Camici et al. 2008). Myocardial hibernation can be partially or completely restored to normal with therapies aimed at improving blood flow such as coronary angioplasty or coronary bypass grafting. Despite sharing important similarities contributing to protection against necrosis, hibernating and preconditioned myocardium differ fundamentally in the expression of mitochondrial electron-transport-chain proteins, which are robustly down-regulated in hibernating hearts (Cabrera et al. 2013). Conversely, increased expression of uncoupling protein 2 (UCP-2), complex IV (cytochrome c oxidase), and complex V (ATPase) proteins is seen in heart mitochondria during late IPC, thereby imparting favorable antioxidant effects and a preserved energetic state during flow-limiting ischemia (Cabrera et al. 2012). This is part of the mechanistic adaptation for maintaining myocardial energetics in the setting of reduced availability of oxygen, potentially as a regulated process that coordinates matching of supply and demand. Morphologically, chronic hibernating myocardial segments show depletion of contractile elements and disorganization of cytoskeletal proteins (Vanoverschelde et al. 1997; Frangogiannis et al. 2002a). This depletion and consequent decrease in metabolic demand allows hibernating myocardium to further reduce rates of consumption of oxygen and substrate (Rahimtoola 1996). While initially reversible by increasing coronary perfusion, chronically underperfused hibernating myocardium eventually loses its ability to reverse the down-regulation of electron-transport-chain proteins, despite successful revascularization (Kelly et al. 2011); the hibernating myocardium also experiences progressive cellular damage, tissue inflammation associated with fibrosis, and remodeling, leading to ischemic cardiomyopathy, heart failure, and death (Frangogiannis et al. 2002a, 2002b).

It remains unclear whether the protective states of myocardial hibernation or IPC resemble mammalian torpor from a molecular standpoint. It has long been appreciated that metabolic suppression during torpor is associated with depressed mitochondrial respiration rates (state 3) in isolated mitochondria from the liver of ground squirrels (Martin et al. 1999), and more recently also confirmed in skeletal and cardiac-muscle mitochondria (Brown and Staples 2014). While this could point to a reduction in the expression and functioning of electron-transport-chain proteins similar to that seen in myocardial hibernation, other studies showed preserved state-3 and state-4 mitochondrial respiration both in brain cortex and in left ventricular myocardium between torpid and interbout-euthermic phases (Gallagher and Staples 2013). Studies of cDNA libraries from the hearts of mammalian hibernators revealed a stress-induced upregulation of mitochodrially encoded subunits of the respiratory-chain proteins, including subunit 2 of NADH-ubiquinone oxidoreductase (ND2, complex I) (Fahlman et al. 2000), cytochrome c oxidase subunit 1 (Cox1, complex IV), and ATP synthase 6/8 (complex V), whereas transcript levels of the nuclear-encoded subunits Cox4 and ATP synthase alpha did not change during hibernation (Hittel and Storey 2002). Collectively, these changes in expression more closely resemble the pattern observed in IPC. At the metabolic level however, myocardial hibernation and IPC share characteristics of increased uptake and utilization of glucose and storage of

glycogen (Cabrera et al. 2013), whereas hearts from torpid hibernators display a shift in fuel-use from carbohydrates and proteins toward fatty acids and ketones, as will be further discussed in subsequent sections (Brown and Staples 2014).

Sepsis, dysfunction of multi-organ systems, depression of metabolic rate: parallels to torpor

Another intriguing comparative biological insight can be drawn between the bioenergetic deregulation and hypometabolic state seen in sepsis-induced dysfunction of organs and natural hibernation. Sepsis is defined as exaggerated systemic inflammatory response syndrome (SIRS) to infection that can progress to multi-organ dysfunction syndrome (MODS), including heart failure, acute respiratory distress syndrome, liver failure, kidney failure, hypothermia, and coagulopathy. Although originally thought to represent the body's response to microbial products such as pathogen-associated molecular patterns, it subsequently became evident that SIRS and MODS can occur without an obvious source of infection. The hosts' innate immune surveillance systems can also be activated by endogenous danger signals collectively called damage-associated molecular patterns, which are released during injury to tissues and can trigger a robust SIRS response, MODS, and death. Interestingly, traumatic tissue-injury with release of mitochondrial products (mitochondrial DNA and formyl peptides) leads to innate immune activation and a sepsis-like state. Several unique features characterize sepsis-induced MODS: (1) a disproportionately low degree of cell death (either necrotic or apoptotic) despite severe clinical and biochemical dysfunction of organs (Hotchkiss et al. 1999; Takasu et al. 2013); (2) maintained, or even elevated, levels of oxygen in tissue within the failed organs (Boekstegers et al. 1991; Rosser et al. 1995; Dyson et al. 2011); (3) an overall reduction in oxygen consumption correlating with the degree of severity of the sepsis (Kreymann et al. 1993); and (4) rapid recovery of organ function following resolution of SIRS. Furthermore, human cells incubated *in vitro* in serum from septic patients display a marked depression in mitochondrial respiration (Boulos et al. 2003; Garrabou et al. 2012). This constellation of manifestations has prompted physician-scientists like Singer and others who are involved in critical care to postulate the novel and intriguing paradigm that MODS could potentially represent an evolutionarily conserved, adaptive strategy of metabolic shutdown, akin to hibernation, to reduce requirements for energy in the face of inflammation-induced mitochondrial dysfunction in sepsis (Singer et al. 2004; Singer 2013). If this hypothesis is borne out, then MODS resembles torpor as a strategy to survive limited resources through adoption of a hypometabolic state. However, the process can progress to become maladaptive when sepsis-induced metabolic downregulation and organismal dysfunction become irreversible. Investigating the regulation of biological pathways invoked by natural hibernators during the adaptive, programmed state of torpor could help identify the key mechanisms involved in the switch from reversible to irreversible mitochondrial inhibition and the dysfunction of organs.

Similarities exist between MODS and torpor with regard to reduced mitochondrial oxidation. In sepsis-induced MODS, defective mitochondrial respiration results in organ failure secondary to bioenergetic impairments, the degree of which correlates with the severity of MODS (Brealey et al. 2002). The effects of SIRS on mitochondria are complex and multifactorial—including inhibition, damage, and reduced turnover of mitochondrial protein—and while the precise mechanisms underlying mitochondrial dysfunction remain unclear, inhibition does correlate with concentration of nitrite/nitrate. Nitric oxide inhibits mitochondrial Complex I activity *in vitro*, and the inverse correlation of nitrite/nitrate with mitochondrial function suggests that reactive nitrogen species reduce mitochondrial function, resulting in decreased utilization of oxygen and substrate in MODS (Brealey et al. 2002). Furthermore, decreased expression and function of cytochrome c oxidase (complex IV) have been observed during sepsis in multiple organs including the heart (Levy et al. 2004), a result that is corroborated by consumption of reduced state-3 oxygen during endotoxemia (Fukumoto et al. 2003; Callahan and Supinski 2005a, 2005b). This inhibition is likely reversible initially, given that recovery from MODS without overt damage to organs is well described in sepsis survivors of sepsis. As mentioned before, several studies have been performed on isolated mitochondria from hibernators during torpor and activity, and it is generally accepted that depression of mitochondrial state 3 is observed during mammalian torpor (Martin et al. 1999; Barger et al. 2003). Reports differ on whether torpor diminishes state-4 mitochondrial activity by directly affecting permeability of membranes to protons or via inhibition of upstream generation of substrate; however, it is clear that mitochondrial function is diminished in torpor, and that the prevailing paradigm is that such reversible inhibition allows hibernating animals to link their metabolic

rate to environmental availability of resources. Nevertheless, in stark contrast to observations in sepsis, cytochrome oxidase-1's steady-state levels of expression (both mRNA and protein) were found to be upregulated during torpor in arctic squirrels in multiple tissues including kidney, heart, and brown fat. Moreover, downregulation of cytochrome oxidase-I transcripts was seen in squirrels that failed to hibernate (Hittel and Storey 2002). Unfortunately, this study did not report on cytochrome oxidase activity, which was reduced by almost 60% in liver mitochondria from hibernating ground squirrels in an earlier study (Lerner et al. 1972).

In the context of bioenergetic failure, several interesting parallels can be drawn between myocardial depression during sepsis and that during mammalian hibernation. While similar reductions in cardiac systolic performance are observed under the two conditions, in contrast to sepsis there is increased diastolic relaxation and improved ventricular compliance during hibernation (Nelson et al. 2003). These functional similarities raise the hypothesis that sepsis-associated myocardial depression may represent a prosurvival adaptive change in ventricular function. Furthermore, striking similarities exist between the myocardial response to ischemia (myocardial hibernation) and sepsis-associated myocardial depression (Solomon et al. 1994), including a number of metabolic alterations, such as upregulation of myocardial-specific glucose transporters (GLUT1, GLUT4), enhanced glucose uptake and utilization, and increased deposition of glycogen; such typical features of hibernating myocardium have been identified in the dysfunctional septic heart (Levy et al. 2005). A fundamental difference between myocardial depression during sepsis versus during ischemia, however, is impairment in the utilization of oxygen, but not in its supply, as evidenced by preserved oxygen tension in the dysfunctional septic heart (Hotchkiss et al. 1991). The evidence is inconclusive regarding the ATP content in the septic myocardium, with some studies reporting preserved ATP levels while others indicating decreased high-energy phosphates during endotoxemia. Clearly, preservation of cellular ATP during sepsis does not equate intact mitochondrial function, as cells adapt to hypoxia by downregulating energy requirements, again supporting the notion of a prosurvival response similar to that occurring during hibernation (Budinger et al. 1998). Nevertheless, when injury to an organ is imminent in the clinical setting, attempts are made to reduce global metabolic rate using the limited means available described next.

Clinical strategies to reduce metabolic rate

Current therapeutic strategies to reduce metabolic demand in humans fall into three main categories: (1) therapeutic whole-body cooling after cardiac arrest, (2) hypothermia during cardiac surgery and circulatory arrest, and (3) cooling of explanted grafts intended for transplant.

Therapeutic cooling after cardiac arrest improves outcomes in patients that have return of spontaneous circulation without return of consciousness (Bernard et al. 2002). Patients are cooled to core levels of 32–34°C, modest compared with small mammalian hibernators but comparable to levels in hibernating bears (Toien et al. 2011). Hypothermia causes a reduction in cerebral demand for energy via temperature-dependent reduction in rates of biochemical reactions (Q_{10}), as well as by lowering levels of the excitotoxic neurotransmitter glutamate. Cooling patients is risky, as humans and other large mammals are highly susceptible to fatal cardiac arrhythmia at body temperatures below 24°C (Fedorov et al. 2005), with some patients experiencing adverse effects at much higher temperatures (Mallet 2002). Hypothermia is also associated with defects in coagulation and immunity, leading to undesirable outcomes in victims of sepsis or traumatic injury (Clemmer et al. 1992; Gentilello et al. 1997). Evidence about cooling after other forms of acute ischemic injury such as stroke has remained inconclusive to date, but further studies are forthcoming (Krieger et al. 2001; De Georgia et al. 2004).

Therapeutic cooling is safely and routinely practiced in cardiac surgery using extracorporeal circulation (cardiopulmonary bypass), with core temperatures between 28°C and 33°C (Griepp et al. 1997). Repair of congenital cardiac anomalies and complex aortic surgery that require periods of circulatory arrest, are conducted under *deep* hypothermia (14–20°C) as the primary means for cerebral protection (Ziganshin and Elefteriades 2013). However, ongoing concerns of morbidity associated with deep hypothermic circulatory arrest have prompted the development of alternative neuroprotective strategies such as *moderate* hypothermic (20–28°C) circulatory arrest with adjunctive selective antegrade cerebral perfusion (Tian et al. 2013). More recently, a new strategy for resuscitation of victims of lethal hemorrhagic shock employing *ultraprofound* hypothermia (<10°C) and using cardiopulmonary bypass has been developed, termed emergency preservation and resuscitation (Alam 2012). Fully understanding how small hibernators' hearts can continue to function at

low temperatures could improve safety and protection of organs in these physiologically challenging scenarios (Dobson 2004). Several potential negative side effects of hypothermia in humans such as increased risk of coagulopathy and bleeding (Rajagopalan et al. 2008), fatal thrombosis (Fanashawe et al. 2001), elevated inflammatory response, increased risk of infection, and end-organ dysfunction (Kourliouros et al. 2010), all seemingly well-tolerated by hibernators, will need to be overcome for use of induced hypothermia to become routine in clinical and emergency settings (Alam 2012).

Preserving organs by rapid cooling (to $\sim 4^{\circ}C$) for delayed transplantation is an additional clinical application of hypothermia. Procurement of organs begins by intravascular flushing with ice-cold preservative solution, followed by their transport in an ice cooler to the recipient. Preservative solutions vary in composition but share the common goals of buffering the inevitable acidosis that develops during ischemia, slowing the intrusion of sodium and water into the cell to prevent swelling, chelation of free calcium, and provision of metabolic substrate for the ischemic graft (Guibert et al. 2011; Voigt and DeLario 2013) This has resulted in solutions high in potassium, buffers, chelators, and osmolality to prevent cellular swelling. While this technique represents many decades of research and development, flushing and packing in ice remains a rudimentary approach to solve an unmet clinical demand. Prolonged cold static storage of organs for transplantation leads to tissue damage and dysfunction of the primary graft (Lima et al. 2006), as well as inferior survival of long-term grafts (Salahudeen 2004). A technological improvement of this technique, involving continuous perfusion of the graft with preservative solution using a perfusion pump, has been shown to improve cold-storage time and initial function of the graft after kidney transplant (Moers et al. 2009), but applications to other solid organs including *ex-vivo* perfusion of the lung have entered clinical practice, especially driven by the expansion of extended-criteria donors and by donation of organs after cardiac death (Roman et al. 2013).

All forms of therapeutic cooling operate on the same unifying principle—hypothermia leads to a reduction in metabolic rate—a highly desirable response during periods of diminished supply. However, this cause–effect relationship during clinical ''hypometabothermia'' is opposite to that naturally employed by hibernating animals that first actively lower their rates of metabolic heat production, which then leads to passive cooling (Heldmaier et al. 2004; Karpovich et al. 2009; Toien et al. 2011). Lessons learned from understanding the mechanisms of temperature-independent suppression of metabolism in hibernators may thus provide a different path by which critically ill patients could benefit from enhanced organ protection. This could also facilitate further development of perfusion of isolated organs at near normothermia, as well as augment the protection of organs afforded by evolutionarily conserved stress-responses such as IPC, which seem to have limited efficacy in circumstances associated with organ transplantation, such as brain death and hypothermia.

Hibernation regulatory molecules

A putative signaling molecule, the hibernation induction trigger (HIT) has remained a matter of interest for over three decades (Dawe and Spurrier 1969), as an opioid-like substance found in the serum of hibernating mammals and hypothesized to initiate hibernation. Initial studies demonstrated that, when administered to mammals that were not hibernating, this serum induced a torpor-like hypometabolic state including a lowering of heart rate, body temperature, and oxygen consumption even if given to non-hibernator species (Dawe et al. 1970; Myers et al. 1981; Oeltgen et al. 1982, 1985). Despite efforts by numerous investigators, the precise identity and functions of HIT remain elusive, and serum-transfusion experiments have proven difficult to replicate (Wang et al. 1988). Although the evidence for opioid induction of torpor remains inconclusive, opioids do seem implicated in governing physiological adaptations that lead to increased resistance to hypothermia, ischemia, and reperfusion in hibernation. This is supported by increased expression of delta-opioid receptors in the brain during torpor (Otis et al. 2010), as well as by a number of mechanisms independent of opioid receptors: a synthetic delta-opioid peptide (DADLE), when administered to cells devoid of opioid receptors, localizes both to the cytoplasm and to the nucleus, induces the formation of nuclear bodies also observed in torpid hibernators and, most importantly, results in significantly reduced transcription rates (Baldelli et al. 2006). Furthermore, nanoparticle-mediated delivery of DADLE induces a reversible hypometabolic phenotype *in vitro* (Colonna et al. 2011). Substantial *in vivo* preclinical evidence implicates delta-opioid agonists in improved time of survival and preservation of organs for transplantation (Oeltgen et al. 1996; Bolling et al. 1997; Inuo et al. 2007), in mitigating the effects of ischemic stroke (Borlongan et al. 2009) and in

reducing myocardial ischemia-reperfusion injury following cardiac surgery (Wu et al. 2011).

Other endogenous ligands also have been implicated in entry and arousal from torpor. Recently, adenosine signaling via adenosine A_1 receptors has been linked to torpor-arousal cycles in the arctic ground squirrel (AGS). Seasonal variation in sensitivity of A_1 receptors, coupled with administration of an adenosine-A_1 receptor agonist into the lateral ventricle of the brain was sufficient to induce torpor in AGS, whereas antagonism at the A_1 receptor reliably blocked entry into torpor (Jinka et al. 2011). These results are identifying a role for CNS adenosine receptor ligands as key regulators of torpor-arousal states.

A hibernation-specific protein complex (HP) identified in the blood of hibernating chipmunks has also been hypothesized to be important in regulating the annual rhythms of hibernation (Kondo and Kondo 1992). Produced in the liver, HP levels in cerebrospinal fluid increase and decrease with the onset and termination of hibernation, and treatment with an antibody to HP is associated with a subsequent decrease in the percentage of animals entering torpor (Kondo et al. 2006). The relationship of HP with hibernation in other species of hibernators, however, is not known.

Hydrogen sulfide (H_2S), a gas typically associated with decaying organic matter, has the capacity to produce a hypometabolic state in mice. H_2S is a potent and reversible mitochondrial complex IV (cytochrome c oxidase) inhibitor, and so it is not surprising that it affects metabolism in animals. In addition, H_2S has been shown to inhibit apoptosis and activate pro-survival kinases (RISK pathway), as well as increase antioxidant mechanisms via Nrf2 (Calvert et al. 2010). When carefully administered in a narrow dose range, mice experienced a reversible decline in oxygen consumption and hypothermia, and they entered a state that resembles torpor (Blackstone et al. 2005), which enabled them to survive hypoxic conditions otherwise fatal to untreated mice (Blackstone and Roth 2007). However, the effects of H_2S on the induction of torpor in large animals are conflicting. Administration of inhaled H_2S to pigs or sheep had no effect on body temperature or metabolism (Haouzi et al. 2008), nor was intravenous administration shown to be protective in a pig model of hemorrhagic shock (Takasu et al. 2013). It is unclear whether differences in body mass, regulation of metabolism in larger mammals, or method of delivery can prevent reduction in metabolic rate in response to H_2S.

As hibernation is a complex phenotype comprising a suite of adaptations, it is unlikely to be triggered by a single substance and also unlikely that a single substance taken from a hibernating animal will completely recapitulate the torpor phenotype in a non-hibernating mammal. It is intriguing, however, that even some aspects of torpor are replicated in phylogenetically distant relatives when they are exposed to certain components of the blood of hibernating mammals. This supports the hypothesis that some of the protective aspects of torpor could be pharmacologically triggered in non-hibernators. Also intriguing is the fact that endogenous delta opioid ligands and adenosine have both been implicated in IPC phenomena and in the induction of torpor.

Additional adaptations in hibernation

While energy conserved by the hypometabolic state induced in torpor could be potentially life-saving for critically ill patients, torpor is further associated with a number of key responsive that are protective of organs including: improved fat metabolism, reduced inflammation, and electrophysiologic adaptations to cold. These additional adaptations are triggered by specific environmental pressures faced by individual species and are therefore not shared among all hibernating animals; for example, hibernating black bears display changes in body temperature that would be considered as only mild hypothermia in humans (Toien et al. 2011). Differences in climate, body-to-surface area ratio, and length of the season have forced a plethora of adaptive phenotypes to develop within the group of mammals that conserve energy via torpor. Many of these adaptations individually could be potentially beneficial for specific disease-states associated with ischemia/reperfusion injury, organ transplantation, dyslipidemia and lipotoxicity, and hypothermia. Understanding how these adaptations work to promote survival in hibernators will be critical in allowing us to adapt mammalian physiology to the prevention of injury to organs in clinical medicine.

Adaptations to cold

Cold inhibits critical enzyme function and the production of vital proteins and nucleic acids. Extremes of cold can be physically disruptive to cellular structure by causing rigidity and stickiness of lipid membranes and the formation of ice; conversion of extra-cellular water to ice drastically changes the osmotic environment of the cell leading to intra-cellular desiccation. An ability to impart resistance to damage of human tissues at sub-zero temperatures

would open a new chapter of cryogenics that would have far reaching implications for organ transplantation. Freeze-resistant strategies employed by arctic fish include the synthesis of anti-freeze proteins that prevent the growth of ice crystals in fish in subzero water (Komatsu et al. 1970). Recently, purified anti-freeze proteins have been used to preserve animal organs for transplantation below 0°C, resulting in prolonged time of viability of grafts (Amir et al. 2004, 2005). Insects have developed wide-ranging adaptations to extreme cold, centered around generation and accumulation of cryoprotectant polyhydric alcohols that promote supercooling in intracellular compartments. Some freeze-tolerant insects couple this strategy with generation of ice nucleators that initiate freezing of extra-cellular water at relatively high, subzero temperatures, while protecting delicate intracellular compartments (Clark and Worland 2008). Insects use a combination of anti-freeze molecules (including membrane-bound glycolipids) (Melvin and Andrews 2009; Walters et al. 2011), freeze tolerance, thermal hysteresis, vitrification, and supercooling to survive temperatures as extreme as −100°C (Sformo et al. 2010). Adaptation of these techniques to organ preservation could allow deep cryopreservation for long-term storage. Recently, a rabbit renal graft was cooled to −138°C and preserved through vitrification that allowed it to be rewarmed and transplanted into a recipient animal, where it remained functional (Fahy et al. 2004, 2009). Long-term storage of viable organs for transplant is currently not performed; if such techniques could be developed they would vastly improve safety and availability of organs for transplantation in the face of ongoing shortages.

While the repertoire of mammalian adaptations to extreme cold is not as varied as that of insects, clinically important mechanisms include freeze-resistance by means of supercooling and adaptations to avoid cardiac arrhythmias. During torpor some of these animals maintain body temperature above 30°C, such as the American black bear (Toien et al. 2011), whereas the AGS drop their body temperature to as low as −2.9°C; they can remain in a supercooled state for as long as 3 weeks (Barnes 1989). Hibernation shares many characteristics across distantly related mammals—reduced metabolism, lowered body temperature, and increased use of fat—but adaptations to survive low temperatures are unique to those animals routinely hibernating in cold environments (Carey et al. 2003).

Bears, like most mammals, suffer cardiac electrical disturbances during hypothermia and typically succumb to fatal arrhythmia or asystole at about 18–21°C (Buresh et al. 2010). It thus appears that only hibernators with small body-mass to surface-area ratios and small heart sizes, and who inhabit cold environments, display electrophysiological adaptations that allow for continued cardiac function at extremely low body temperatures. Since the early experiments using hypothermia during surgery, it has become clear that non-hibernating mammals lack adaptations for sustained cardiac function at low organ temperatures (Bigelow et al. 1954; Bigelow 1984). While hypothermia offers a measure of organ protection by secondarily lowering metabolic rates, body temperatures below 28°C are pro-arrhythmic and unless there is extracorporeal circulatory support, life-threatening cardiac arrhythmias often develop (Polderman and Herold 2009). Thus, the clinical advantages of organ protection yielded by extreme hypothermia are limited by the additional morbidity of cardiopulmonary bypass. The reduction in heart rate that is characteristic of entrance into torpor, like the associated global reduction in metabolic rate, appears to be independent of body temperature and precedes any changes in it (Elvert and Heldmaier 2005). Forced induction of hypothermia in Syrian hamsters induced J-waves and atrioventricular block while spontaneous hibernation had no adverse effect (Miyazawa et al. 2008), further supporting the concept that entrance into hibernation is a highly regulated event of several physiological processes, rather than merely a consequence of reduced body temperature. Small hibernating animals have intrinsic electrophysiological properties and adaptations allowing the maintenance of regular cardiac rhythm, despite body temperatures near 0°C (Burlington and Milsom 1993; Johansson 1996; Milsom et al. 1999; Wang and Zhou 1999; Fedorov et al. 2005). A primary adaptation is the ability to maintain cellular membrane potential at low tissue temperatures. Normal resting potentials of cardiac membranes are −80 to −90 mV at 37°C, but with hypothermia they fall to approximately −45 mV in non-hibernating mammals, thereby decreasing the depolarization threshold and often resulting in ventricular fibrillation and other fatal arrhythmias (Wang et al. 2002). Conversely, hibernating mammals are able to defend their membrane potential close to normal at low temperatures, with cardiac myocyte membrane potential in AGS remaining near −60 mV despite subfreezing body temperatures (Wang and Zhou 1999). This remarkable feat is achieved through improved handling of electrolytes at low temperature, predominantly Ca^{2+} and Na^{+}. The cold-adapted phenotype consists of reduced expression and activity of L-type voltage-gated Ca^{2+}

channels, voltage-gated Na^+ channels, and decreased activity of the Na^+/Ca^{2+} exchanger at low temperatures, further reducing the risk of total depolarization of membranes. When coupled with an increased quantity of sarcoplasmic reticulum to compensate for the reduced calcium influx from the plasma membrane, and an upregulation of connexins (Fedorov et al. 2005), small hibernators are able to preserve cardiomyocyte contractility and avoid arrhythmias at low organ temperatures by slowing the velocity of ventricular conduction and increasing the excitation threshold (Alekseev et al. 1996; Kokoz et al. 1997). During arousal, hibernators display a waxing and waning cardiac rhythm, with an initial increase in heart rate followed by periods of asystole and profound bradycardia between 11°C and 18°C, finally attaining a regular rhythm at about 18°C (Eagles et al. 1988), all thought to be determined by alternating sympathetic versus parasympathetic dominance until the animal reaches euthermia (Milsom et al. 1999). Similarly, strong autonomic control of heart rate can be seen in large hibernators. Black bears display extreme variations in heart rate (respiratory sinus arrhythmia), with periods of asystole as long as 14 s during hibernation and in the months preceding hibernation, which typically indicate a predominance of vagal (parasympathetic) tone of the heart. However, in pregnant bears heart rates continue to increase as pregnancy continues, spike during birth, then promptly return to hibernation levels, suggesting cardiac sympathetic control is also preserved during hibernation (Laske et al. 2010). Understanding the mechanisms and physiological role of respiratory sinus arrhythmia in large hibernators is important, especially given increasing evidence that vagal afferent stimulation exerts cardioprotective effects in ischemia-reperfusion (Shinlapawittayatorn et al. 2013) as well as anti-inflammatory effects in sepsis (Borovikova et al. 2000).

In contemporary cardiac surgery two primary methods are utilized to protect the heart, both offering parallels with hibernation biology: (1) hypothermia with the aid of extracorporeal circulation and (2) cardioplegia, a pharmacologic treatment that stops cardiac activity during surgery to reduce consumption of myocardial oxygen and substrate. Traditional cardioplegia is based on a solution with a high concentration of potassium that causes rapid electrochemical arrest through complete depolarization of the plasma membrane, and thus lowers myocardial oxygen consumption. *Depolarizing cardioplegia* maintains intracellular and extracellular potassium concentrations at near equilibrium; therefore, the cell cannot regain its membrane potential until the solution is washed away. Cold depolarizing hyperkalemic cardioplegia has been associated with deleterious consequences including increased endothelial inflammation, superoxide production, platelet aggregation, coronary vasoconstriction, and altered distribution of myocardial blood flow, increased Na^+/Ca^{2+} exchanger activity, and myocyte intracellular Ca^{2+} overload (Hearse et al. 1993; Vinten-Johansen 2004). This may in turn cause post-reperfusion arrhythmias, stunning, inflammation, necrosis, and apoptosis, yet cold depolarizing cardioplegia remains the most commonly used method for surgical myocardial protection worldwide. An alternative strategy involves inducing cardiac arrest by polarizing the plasma membrane, an example of which has been popularized by Dobson and Vinten-Johansen (Corvera et al. 2005; Sloots et al. 2007). Using a normokalemic solution of adenosine—lidocaine—magnesium, these investigators demonstrated effective cardiac arrest with potentially superior cardioprotection (Letson and Dobson 2011a; Shi et al. 2012). Of particular relevance to this audience, the physiological basis for developing *polarizing cardioplegia* has been informed by lessons from natural hibernators, who (1) reduce their myocardial metabolism without imposing a depolarizing insult on their cardiomyocytes, and (2) preserve coronary blood flow despite the low-energy state of the heart in torpor (Dobson 2004; Dobson et al. 2013). Adenosine opens the K_{ATP} channels, thereby shortening the duration of the action potential in the atria, Purkinje fibers, and ventricles; lidocaine blocks the inward Na^+ fast-current channels and thus reduces the amplitude of the voltage of the cardiac action potential to its diastolic baseline—the combination resulting in electrochemical cardiac arrest by ''clamping'' the membrane potential at its resting diastolic value of −80 to −85 mV (Dobson 2004), similar to the electrophysiological adaptations found in the hearts of hibernators. Additional benefits of adenosine-lidocaine are a rapid and reversible reduction of heart rate; a slowing of atrioventricular conduction; and coronary vasodilatory, anti-ischemic, anti-arrhythmic, and possible coagulative correcting effects (Dobson et al. 2013). The fundamental advantage of polarized arrest and reanimation stems from fewer membrane channels and pores being opened and exchangers being activated, compared with the depolarized state; depolarized cells are electrically more unstable, whereas maintaining a polarized membrane potential during global or regional ischemia is a key factor in preventing the endothelium from triggering a host of local immunologic and coagulopathic derangements

(Dobson and Jones 2004; Ward et al. 2006). Following a large number of successful preclinical studies, including one demonstrating improved post-ischemic recovery after 8 h of static cold storage (Rudd and Dobson 2011), polarizing cardioplegia with adenosine-lidocaine-magnesium was tested in randomized controlled trials and proven to be superior to depolarizing hyperkalemic cardioplegia both in adult (Onorati et al. 2013) and pediatric patients (Jin et al. 2008). Furthermore, applications of adenosine-lidocaine-magnesium at lower concentrations have been proposed as a biological response modifier in the setting of resuscitation from trauma and severe hemorrhagic shock (Letson and Dobson 2011b), in part due to its coagulation restorative (Letson et al. 2012) and anti-inflammatory properties (Shi et al. 2012).

Metabolic switch: a preference for fatty-acid oxidation

A genomic and biochemical reprograming fundamental to hibernation is the metabolic switch to virtually exclusive use of fatty acids as metabolic fuel during steady-state torpor. Many aspects of this adaptation are intuitive: fat is energy dense and an efficient form of energy storage, fat catabolism generates metabolic water, and fat stores may also provide thermal insulation during environmental temperature extremes. However, if torpor is viewed as the body's adaptive response to stress from environmental austerity, then the metabolic switch to fatty-acid oxidation stands in contrast to how tissues handle other forms of physiologic stress.

The healthy heart is considered a metabolic omnivore, maintaining the ability to derive energy from fat, carbohydrate, amino acids, or ketones (Goodwin et al. 1998), with fatty-acid oxidation representing the predominant metabolic fuel under normal resting conditions (Bing et al. 1954). However, during mechanical, ischemic/hypoxic, or inflammatory stress, the heart will transition to the metabolism of increasing amounts of carbohydrates (Taegtmeyer 2002). Generally, these changes are adaptive, allowing the heart to utilize the most efficient and abundant substrate for a given set of environmental conditions. Short-term changes may simply take place at the level of enzyme activity in the heart, but long-term changes require altering the metabolic machinery and appear to involve the peroxisome proliferator-activated receptor family of nuclear receptor proteins. These master regulators of metabolism are responsible for changing the expression level of key metabolic enzymes involved in metabolism and making lasting changes in cellular bioenergetics (Kelly 2003).

In the non-hibernator heart under continued hypoxic stress, transcriptional changes result in reduced metabolic flexibility; enzymes involved in the oxidation of fatty acids are downregulated, and the heart transitions to carbohydrate metabolism (Razeghi et al. 2001). Myocardial hibernation is associated with such switch from fatty acids to glucose as the preferred metabolic substrate, which provides the rationale for using radionuclide myocardial perfusion imaging with fluorine-18 labeled glucose to identify viable but hibernating myocardial segments in patients. Although this switch in fuel teleologically may occur because carbohydrate metabolism requires less oxygen than does oxidation of highly reduced fatty acids, it is in stark contrast with the metabolic changes observed in hibernators. These animals increase expression of enzymes involved in fatty-acid oxidation while reducing their capacity for glycolysis in preparation for seasonal hibernation (Yan et al. 2008; Shao et al. 2010). Certainly in the AGS, torpor-arousal cycles would likely result in periods of relative ischemia, especially during rewarming and interbout arousal, intervals that occur each 1–3 weeks during the hibernation season. If the hibernator's heart were to be viewed as a natural model for tolerance to stress, then the switch to carbohydrate metabolism made by non-hibernators would be construed as maladaptive. Non-hibernators may not be capable of continuing oxidation of fatty acids in times of physiologic stress. Without the hibernator's adaptations for improved metabolism of fatty acids, non-hibernators experience significant toxicity associated with fatty-acid oxidation in times of stress, by developing metabolic bottlenecks (Turer et al. 2009, 2010). Incompletely oxidized fatty acids build up in the form of ceramides and acyl-carnitines (Zhou et al. 2000; Unger and Orci 2001), thereby triggering further inflammation and cell-death. If more efficient processing of fatty acids during times of stress were possible in non-hibernators, their bioenergetics phenotype would more closely approximate that of an unstressed heart. While it remains unclear if this would in fact be metabolically advantageous in humans, it clearly has been a successful strategy for diverse species of hibernating mammals during times of low perfusion and low availability of resources.

Immunological and hematological adaptations relevant to organ protection

Torpor is also associated with suppression of both the innate and adaptive immune system that appears

to be obligatory for surviving torpor-arousal cycles. This closely resembles the compensatory anti-inflammatory response syndrome that develops in severe sepsis, a state of immunoparalysis often characterized by secondary infections with opportunistic organisms that are otherwise uncommon pathogens for immunocompetent hosts. Early observations in hibernators demonstrated that torpor is associated with a reliable and dramatic reduction in circulating leukocytes. Leukopenia with approximately 90% reduction in circulating white blood cells (WBCs) appears to be a common feature across several species of hibernating mammals during torpor (Szilagyi and Senturia 1972; Spurrier and Dawe 1973; Suomalainen and Rosokivi 1973; Reznik et al. 1975; Frerichs et al. 1994; Toien et al. 2001; Bouma et al. 2010; Sahdo et al. 2013). The reduction in WBC during torpor primarily affects granulocytes, lymphocytes, and monocytes. The remnants of circulating WBCs are 90% neutrophils and 9% lymphocytes. Interestingly, cell numbers rapidly (within 1.5 h) recover during arousal from torpor, neutrophils recovering to summer values, and lymphocytes to ~50% of summer values within ~24 h (Suomalainen and Rosokivi 1973). These findings argue against the hypothesis that leukocytes undergo massive apoptosis in torpor and are newly synthesized during arousal, suggesting that other mechanisms for retention are involved and appear to be immune-cell-type specific. Neutropenia during torpor seems to be entirely driven by hypothermia-induced reversible margination of cells, as it is not affected by splenectomy and is abolished by pretreatment with dexamethasone. This temperature-induced transient neutropenia is not restricted to deep hibernators, as it also occurs in daily torpor as well as in non-hibernating species during forced hypothermia (Bouma et al. 2013a). On the other hand, lymphopenia during torpor is due to hypothermia-induced retention of cells in peripheral lymphoid organs, which is regulated via altered plasma levels of sphingosine-1-phosphate (Bouma et al. 2011). Again, this response appears widely conserved in mammals (shared by hibernating hamsters, hypothermic hamsters, and hypothermic non-hibernating rats).

The adaptive value of leukopenia in the setting of ischemia-reperfusion is suggested by several clinical studies in cardiac surgical patients. These studies identified preoperative high WBC count as a risk factor for stroke, atrial fibrillation, myocardial infarction, and 1-year mortality, whereas leukocyte depletion in the same setting results in improved organ function (Gu et al. 1996; Albert et al. 2003; Newall et al. 2006). Furthermore, since neutrophil activation, margination, and transmigration are essential in the pathophysiology of organ injury following ischemia-reperfusion and hypothermic cardiopulmonary bypass, additional comparative biological studies could elucidate the mechanisms by which hibernators avoid organ injury despite regularly cycling through periods of extreme hypothermia followed by rapid rewarming. While circulating WBCs decrease during torpor, certain compartments including the intestine, lungs, liver, and spleen, sequester WBCs and experience a local increase in cell number (Inkovaara and Suomalainen 1973; Yasuma et al. 1997). Neutrophils are reported to increase at the lung epithelial barrier (Inkovaara and Suomalainen 1973), whereas the intestinal epithelial barrier experiences predominantly an increase in lymphocytes (Kurtz and Carey 2007)—likely representing protective responses at sites of exposure to potential pathogens during the targeted immunosuppression of torpor. Hibernators also experience thymic involution, reduced production of bone marrow, and thymic maturation of lymphocytes during torpor (Galletti and Cavallari 1972; Novoselova et al. 2004). Given the long life of naïve lymphocytes, this only accounts for part of the observed reduction in circulating lymphocytes (Sprent and Tough 1994; Parretta et al. 2008), with the remainder sequestered in lymphoid organs.

In addition to reducing the numbers of circulating WBCs, torpor changes WBC's activity in response to insult. LPS binding to splenic macrophages appears to be unaffected by arousal cycles (Maniero 2005). However, production of tumor necrosis factor alpha in response to LPS is significantly reduced in peritoneal macrophages taken from hibernating animals, even under normothermic conditions (Novoselova et al. 2000a, 2000b). This hibernation-induced immune unresponsiveness (i.e., anergy) *in vitro* translates to phenotypic differences *in vivo,* as torpid ground squirrels do not mount a febrile response to peritoneal injection of LPS, although fever can be observed during the next arousal (Prendergast et al. 2002). The preserved ability to bind LPS, coupled with a reduction in cytokine production, suggests that CD14 expression on innate immunocytes is intact during torpor but has a reduced capacity to heterodimerize with TLR4, or that there is a reduction in the signaling capacity downstream of the PRRs. In an intestinal model of warm I/R injury, elevated levels of myeloperoxidase were seen following I/R injury in ground squirrels in summer but not when torpid (Kurtz et al. 2006). This suggests that while equivalent ischemic organ-injury took place, torpid squirrels failed to recruit neutrophils into

the injured organ, thus sparing them injury from reperfusion. The mechanisms underlying the reduced humoral immune function during hibernation are also beginning to be elucidated. Recent studies have revealed that T-cell-independent humoral immune responses were specifically impaired in torpor, possibly due to reduced levels of plasma complement. Conversely, the response to T-cell-dependent antigens was preserved, and was associated with disturbance of torpor-behavior and increased mortality (Bouma et al. 2013b). Although generally thought to be an adaptive response, the immunosuppressed state that occurs during torpor can put hibernating animals at risk for opportunistic infections such as the deadly white-nose syndrome described in hibernating populations of bats (Cryan et al. 2010). As the complex immunomodulation occurring in torpor is better understood, translational applications to severe infection in humans may become apparent and may allow development of effective therapeutics in the face of limited metabolic resources.

Furthermore, studies of the blood-cell dynamics of hibernating mammals have identified drastic 10-fold reductions in circulating thrombocytes during torpor, rising rapidly to near-normal levels upon return to euthermia (Pivorun and Sinnamon 1981; Bouma et al. 2010). Also, studies of platelet aggregation identified significant decreases in platelet function in brown bears compared with humans (Frobert et al. 2010). Mammalian hibernators greatly decrease circulating levels of clotting factors VIII and IX during torpor (Pivorun and Sinnamon 1981), while increasing their levels of alpha2-macroglobulin and haptoglobin (Srere et al. 1995; Carey et al. 2003; Mominoki et al. 2005). These mechanisms combine to increase clotting time during hibernation; likely as an adaptive response to prevent thromboembolic events despite highly thrombogenic states associated with reduced blood flow, stasis, hypothermia, and prolonged immobility.

Strategies for organ protection

Torpor is a complex phenotype consisting of (1) profound whole-animal hypometabolism, (2) obligate oxidation of fatty acids during torpor, and (3) an immunosuppressed state. This phenotype has developed over millions of years of evolution and is quite similar in phylogenetically distant species of mammals. This similarity could arise from conservation of genes involved in torpor from a common ancestor or convergence of species onto a single successful strategy. The wide variety of species that utilize torpor to balance supply-and-demand relationships suggests that some of these mechanisms may be able to be adapted to the human condition. Supply-and-demand relationships often are in delicate balance in critical-care and perioperative medicine. The majority of clinical treatments are focused on the supply end of the equation, while a paucity of therapies are able to decrease demand. Adaptation of molecular mechanisms unearthed in the study of hibernation may open a new chapter in how critically ill patients are managed; by spending less we may be able to accomplish more.

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