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REVIEW

# Adaptations to a hypoxic lifestyle in naked mole-rats

**Matthew E. Pamenter***


## ABSTRACT

Hypoxia is one of the strongest environmental drivers of cellular and physiological adaptation. Although most mammals are largely intolerant of hypoxia, some specialized species have evolved mitigative strategies to tolerate hypoxic niches. Among the most hypoxia-tolerant mammals are naked mole-rats (*Heterocephalus glaber*), a eusocial species of subterranean rodent native to eastern Africa. In hypoxia, naked mole-rats maintain consciousness and remain active despite a robust and rapid suppression of metabolic rate, which is mediated by numerous behavioural, physiological and cellular strategies. Conversely, hypoxia-intolerant mammals and most other hypoxia-tolerant mammals cannot achieve the same degree of metabolic savings while staying active in hypoxia and must also increase oxygen supply to tissues, and/or enter torpor. Intriguingly, recent studies suggest that naked mole-rats share many cellular strategies with non-mammalian vertebrate champions of anoxia tolerance, including the use of alternative metabolic end-products and potent pH buffering mechanisms to mitigate cellular acidification due to upregulation of anaerobic metabolic pathways, rapid mitochondrial remodelling to favour increased respiratory efficiency, and systemic shifts in energy prioritization to maintain brain function over that of other tissues. Herein, I discuss what is known regarding adaptations of naked mole-rats to a hypoxic lifestyle, and contrast strategies employed by this species to those of hypoxia-intolerant mammals, closely related African mole-rats, other well-studied hypoxia-tolerant mammals, and non-mammalian vertebrate champions of anoxia tolerance. I also discuss the neotenic theory of hypoxia tolerance – a leading theory that may explain the evolutionary origins of hypoxia tolerance in mammals – and highlight promising but underexplored avenues of hypoxia-related research in this fascinating model organism.


**KEY WORDS: Glycolysis, Heterotherm, Hypoxia, Hypoxic metabolic response, Hypoxic ventilatory response, Ischaemia, Mammals, Metabolic rate suppression, Mitochondria, Thermoregulation**

## Introduction – living la vida hypóxica

Hypoxic environments are common in nature but despite the energetic challenge associated with life in low oxygen, animals have spread into every hypoxic niche on Earth (Bickler and Buck, 2007; Childress and Seibel, 1998; Storz and Scott, 2019). For terrestrial vertebrates, hypoxia is a challenge faced by animals that inhabit densely populated underground burrows or caves (e.g. African mole-rats, bats), dive to forage (e.g. various pinnipeds) or dwell in high-altitude niches (e.g. *Peromyscus maniculatus* – high altitude deer-mice, *Eospalax fontanierii baileyi* – plateau zokors), among others (McClelland and Scott, 2019; Storz et al., 2010; Tian et al., 2016). For such species, hypoxia has acted as a strong selective force that has driven the evolution of cellular and physiological traits that enhance tolerance to low-oxygen stress (Dzal et al., 2015; Pamenter et al., 2020; Storz and Scott, 2019).

The energetic challenge of hypoxia manifests primarily at the mitochondrial level but has major ramifications across all levels of physiology (Galli and Richards, 2014; Pamenter, 2014). The key to tolerating hypoxia is to match metabolic demand to reduced energy supply (Boutilier, 2001; Buck and Pamenter, 2006; Hochachka, 1986; Hochachka et al., 1996). In general, most adult hypoxia-tolerant animals prioritize reducing energy demand, exhibiting robust metabolic rate suppression (see Glossary) when oxygen is limited (Guppy and Withers, 1999); however, there are notable exceptions among species that perform energy-intensive behaviours underwater or at altitude and employ different strategies (Dzal et al., 2015; McClelland and Scott, 2019; Scott et al., 2015; Tian et al., 2016). Conversely, hypoxia-intolerant animals are generally unable to sufficiently suppress metabolic demand during hypoxia to match reduced oxygen availability; instead, they increase ventilation in a largely futile attempt to maintain normal oxygen delivery. This is an energetically expensive strategy, particularly when breathing air with reduced oxygen content (Hochachka, 1986; Pamenter and Powell, 2016). As a result, and in the absence of sufficient metabolic rate suppression, most mammals rely (to varying degrees) on anaerobic energy production to make up the gap between energy demand and reduced aerobic energy production. This is, at best, a short-term adaptive strategy because it leads to lactate accumulation and cellular acidification, which, assuming the organism can tolerate the duration of the hypoxic exposure, must be metabolized upon reoxygenation.

Over the last several decades, we have made major advances in our understanding of the physiological and cellular adaptations to hypoxia of several organisms that have demonstrable hypoxia or anoxia tolerance; however, this information is relatively ‘siloed’ as many researchers explore specific niche areas within their chosen species of study. This is particularly true of the study of terrestrial mammalian models of hypoxia tolerance, which have historically received comparatively little attention relative to more thoroughly studied hypoxia- (or anoxia)-tolerant fish, amphibians and reptiles (Bickler and Buck, 2007). As a result, we have little insight into several key questions in this field. For example, we have limited knowledge regarding the developmental and evolutionary origins of these traits in mammals, and the impact that concomitant environmental pressures (e.g. cold, hypercapnia, etc.) and/or sociality have on shaping physiological responses to hypoxia. In this Review, I discuss common strategies of adaptation in hypoxia-tolerant mammals, with a particular focus on one of the most hypoxia-tolerant mammals – naked mole-rats (NMRs; *Heterocephalus glaber*, Ruppell 1842). In addition, I contrast these adaptive traits with better-described strategies in anoxia-tolerant vertebrates, and discuss the neotenic theory of hypoxia tolerance,

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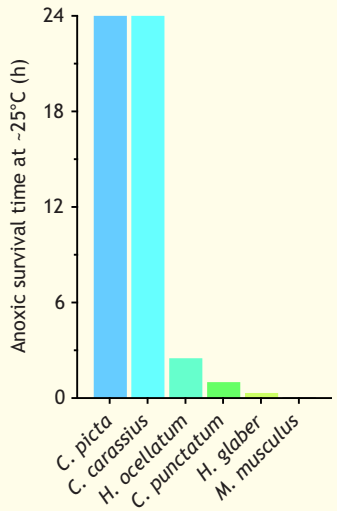
## Glossary

**Anapyrexia**
A regulated decrease in body temperature below normal.
**Dead-space ventilation**
The volume of air that is inhaled but does not take part in gas exchange because it remains in conducting airways or reaches alveoli that are poorly perfused.
**Fossorial**
Species that live primarily underground.
**Heterotherm**
An organism whose ability to regulate its body temperature is intermediate between endothermy (physiological regulation of body temperature) and ectothermy (environmental regulation of body temperature.
**Hypoxic metabolic response**
A change in metabolic rate mediated by hypoxia.
**Hypoxic ventilatory response**
A reflex increase in ventilation mediated by hypoxia.
**Ischaemia**
Reductions in both oxygen and nutrient availability, usually due to reduced or occluded local blood flow.
**Ischaemia–reperfusion injury**
Damage caused when blood supply returns to a tissue after a period of ischaemia.
**Metabolic rate suppression**
A decrease in metabolic rate in response to environmental stress.
**Non-shivering thermogenesis**
An increase in metabolic heat production that is not associated with muscle activity.
**Oxygen transport cascade**
The series of physiological systems that transports oxygen from inspired air to tissues, including ventilation, diffusion and perfusion.
**$P_{50}$**
The oxygen tension at which haemoglobin is 50% saturated.
**Proteostasis**
The controlled maintenance of protein genesis, folding, trafficking and degradation by competing and integrated biological pathways.
**Tidal volume**

## Box 1. What is hypoxia tolerance?




Historically, hypoxia has been defined from an anthropocentric viewpoint as being less than the atmospheric oxygen concentration near sea level that most humans experience (~21% $O_2$); however, this has limited utility in the study of animals for whom this might be considered hyperoxic. Indeed, there is no broadly accepted definition of 'hypoxia tolerance' or even of 'hypoxia' in the literature. As a result, there is often debate regarding which animals are the most hypoxia tolerant and what exactly constitutes tolerance to hypoxia. For the purposes of this Review, I define hypoxia as being a level of ambient oxygen that is (a) not sufficient to meet an organism's basic physiological requirements, and (b) requires some degree of adaptation to tolerate. Note that this definition of hypoxia is subjective to the individual species. Conversely, anoxia is the absence of any oxygen and is thus the most severe natural low-oxygen challenge an animal can face. This figure compares the survival time of various species in anoxia at a common temperature of ~25°C (Chapman et al., 2011; Nilsson and Renshaw, 2004; Park et al., 2017; Ramaglia and Buck, 2004). Note that, although naked mole-rats can survive for 18 min in anoxia, which is remarkable for a mammal, this ability pales in comparison to that of the freshwater turtle, *Chrysemys picta*, and the crucian carp, *Carassius carassius*, both of which can survive in anoxia for >24 h at this temperature.

which is a leading hypothesis regarding the evolutionary origins of hypoxia tolerance in mammals. Finally, I discuss the translational potential of the study of hypoxia-tolerant species to inform biomedical questions.

### Naked mole-rats – mammalian champions of hypoxia tolerance

NMRs are among the most hypoxia-tolerant mammals identified. In the laboratory, NMRs tolerate <3% $O_2$ for several hours (Pamenter et al., 2014b, 2019, 2018), 8–10% $O_2$ for days to weeks (Chung et al., 2016), and anoxia for up to 18 min (Park et al., 2017). Comparatively, a typical 'normoxic' atmosphere at sea level contains ~21% $O_2$, and >15 min of <5% $O_2$ is lethal to mice (Park et al., 2017). Importantly, although remarkable for a mammal, the ability of NMRs to tolerate a few minutes of anoxia pales in comparison to animals that are truly anoxia tolerant and survive without oxygen for days at room temperature (see Box 1), or weeks to months in colder temperatures (Bickler and Buck, 2007). NMRs should not be considered anoxia tolerant but are more accurately able to resist the lethal effects of anoxia for a very short time. In this way, they might be better labelled as 'slow-diers', like the leopard frog, *Rana pipiens* (Milton et al., 2003), in which metabolic rate suppression and cytoprotective regulation of excitatory neurotransmitters effectively delay, but do not prevent, death in anoxia.

The tolerance of NMRs to low oxygen was long assumed to be the result of adaptation to a putatively constant hypoxic atmosphere in NMR burrows. Indeed, it was assumed that in the wild, and given their deep nests and the large number of animals within each colony, that NMRs most likely encounter chronic hypoxia throughout their lives (Larson et al., 2014). However, recent measurements from NMR burrows call this supposition into question and instead suggest that burrow oxygen levels are only slightly hypoxic relative to atmospheric conditions (Holtze et al., 2018). Notably though, measurements in this study were taken from tunnel regions near freshly excavated burrow openings and at relatively shallow depths, limiting the conclusions that can be drawn from these data. Nonetheless, NMRs likely do experience periods of hypoxia at their metabolic extremes: both within their crowded and poorly ventilated nest chambers while resting and while working to their physical limits while digging and excavating with their noses pressed into the ends of vast tunnel networks, rebreathing previously expired air. However, NMR tunnel networks often span many kilometres (Brett, 1991), and it is therefore highly likely that the majority

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of these networks are not densely populated or particularly hypoxic. Therefore, NMRs may experience severe hypoxia, but intermittently and interspersed with periods of relative normoxia [i.e. approaching surface (atmospheric) oxygen levels; Buffenstein et al., 2022]. In this fashion, NMRs likely encounter irregular cycles of hypoxia–reoxygenation in their day-to-day lives, which is a relatively rare environmental challenge for a terrestrial mammal but is of particular interest to biomedical questions related to ischaemia–reperfusion (see Glossary and below).

The mechanisms underlying the remarkable hypoxia tolerance of NMRs received surprisingly little attention until ∼10 years ago, with the exception of the key finding that they possess a high-affinity haemoglobin isoform that is similar to that of neonatal mammals (Johansen et al., 1976). This finding has been recently extended to several social and solitary cousin species of NMRs (Weber et al., 2017). As a result, fossorial African mole-rats have a neonatal haemoglobin $P_{50}$ (see Glossary) and thus, in hypoxia, their tissues are presumably perfused with blood containing a relatively higher oxygen level than would be observed in non-adapted species breathing the same atmospheric gas concentration. More recently, several studies have begun to describe physiological and cellular adaptations that are beneficial to life in hypoxia in NMRs and, to a lesser degree, in related mole-rat species. These adaptations span from the behavioural to the biochemical level, suggesting that a wide range of strategies contribute to metabolic savings during hypoxia in these animals.

## Achieving metabolic balance – suppressing energy demand in hypoxia

Most adult mammals do not exhibit metabolic rate suppression in hypoxia (Frappell et al., 1992; Pamenter and Powell, 2016). Conversely, metabolic rate suppression is commonly observed in many of the most hypoxia-tolerant vertebrates (Bickler and Buck, 2007; Dzal et al., 2015; Guppy and Withers, 1999; Pamenter et al., 2015; Walsh et al., 1996), and in neonatal mammals (Dzal et al., 2020; Frappell et al., 1992, 1991), which are generally more hypoxia tolerant than their adult counterparts (Singer, 1999). Early measurements of the hypoxic metabolic response (see Glossary) in NMRs suggested that these animals, like most other adult mammals, do not reduce their metabolic rate substantially in hypoxia (Nathaniel et al., 2012); however, issues with the experimental design employed in this study (including the use of a large experimental chamber and high gas flow rates) likely masked the true metabolic response to hypoxia. There is now robust evidence supporting the hypothesis that NMRs rely strongly on metabolic rate suppression as their primary adaptive response to hypoxia. For example, at the whole-animal level, NMRs suppress metabolic rate by up to 85% in severe hypoxia (3% $O_2$; Fig. 1A; Pamenter et al., 2014b; Pamenter et al., 2019), and this suppression conforms to oxygen availability, such that metabolism decreases proportionally with decreasing oxygen availability (Pamenter et al., 2019). The mechanisms underlying this metabolic suppression are not fully elucidated, but experiments to date suggest energy savings are

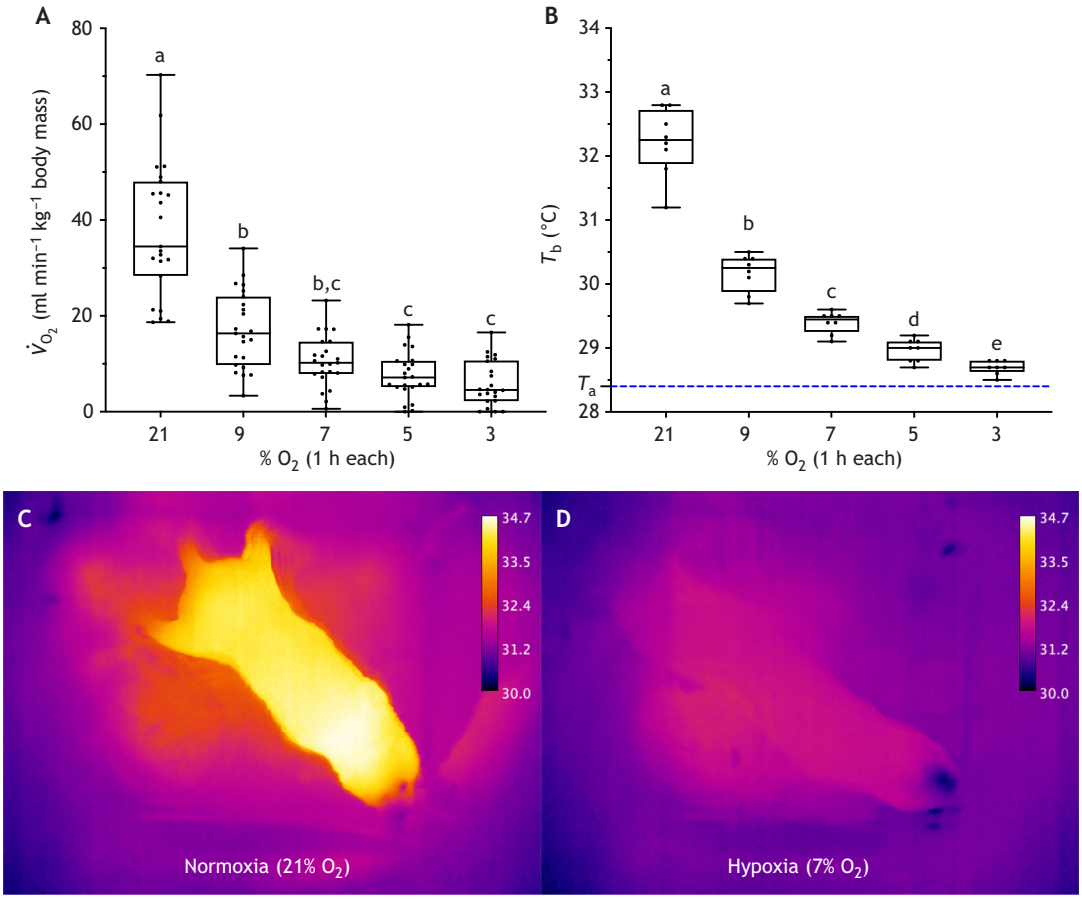


**Fig. 1. Naked mole-rat (NMR) metabolic rate and thermogenesis decrease in acute hypoxia and body temperature drops to ambient levels.** (A,B) Summary of oxygen consumption ($\dot{V}_{O_2}$; A) and body temperature ($T_b$; B) from NMRs exposed to progressively deeper hypoxia at ∼28°C. Data are means±s.e.m. (repeated measures ANOVA with Tukey *post hoc* test). Different letters indicate statistical difference ($P$<0.05). (C,D) Thermal images of a NMR following 60 min of exposure to normoxia (21% $O_2$; C) or hypoxia (7% $O_2$; D) at ∼30°C. Adapted from Pamenter et al. (2019) and Cheng et al. (2021b).

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achieved at least in part from rapid reductions in activity and thermogenesis in hypoxia (Figs 1 and 2), and via a suite of adjustments in cellular and organelle activity and function (see below).

### Behavioural adaptations to hypoxia and social influences

Many small mammals enter a torpor-like state in hypoxia and remain in this state until more favourable oxygen conditions are available; however, this strategy is clearly not optimal for NMRs, which experience hypoxia in their crowded nests where oxygen availability is unlikely to spontaneously increase. Indeed, as it was previously generally assumed that NMRs live in a chronically hypoxic environment it was thought that their behaviour must be unaffected by reduced oxygen levels. However, quantitative evaluation of the impact of hypoxia on NMR behaviour revealed that NMRs do decrease overall activity (including time spent active and movement velocity) when acutely exposed to 7% $O_2$ or lower, and that these changes become enhanced with progressively deeper hypoxia (Fig. 2A,B; Ilacqua et al., 2017; Kirby et al., 2018). However, despite marked decreases in metabolic rate and body temperature ($T_b$), NMRs do not cease activity, lose consciousness or enter a torpor-like state in even the most severe levels of hypoxia tested (3% $O_2$).

Intriguingly, this behavioural retardation in hypoxia is largely absent when NMRs are tested in pairs or in groups (Fig. 2C; Houlahan et al., 2018), suggesting that social interactions supersede the need to reduce activity in hypoxia in this eusocial species, and also that metabolic savings from decreased activity may not be critical to tolerating hypoxia. Alternatively, reductions in metabolic costs and evaporative water loss associated with huddling in pairs or groups may confer sufficient energy savings to mitigate the need for reduced activity in hypoxia, particularly as the NMR dermis has poor barrier characteristics against evaporative water loss (Menon et al., 2019). However, exposure of groups of NMRs to acute hypoxia (7% $O_2$) does not affect huddling behaviour (Houlahan et al., 2018), suggesting that it is social imperatives and not energy savings associated with huddling that drive the maintenance of normal physical activity and exploratory behaviour in groups of hypoxia-exposed NMRs. The social aspects of physiological responses to hypoxia have been largely overlooked (in this species and elsewhere), and this is an interesting area that is ripe for further exploration within a comparative framework.

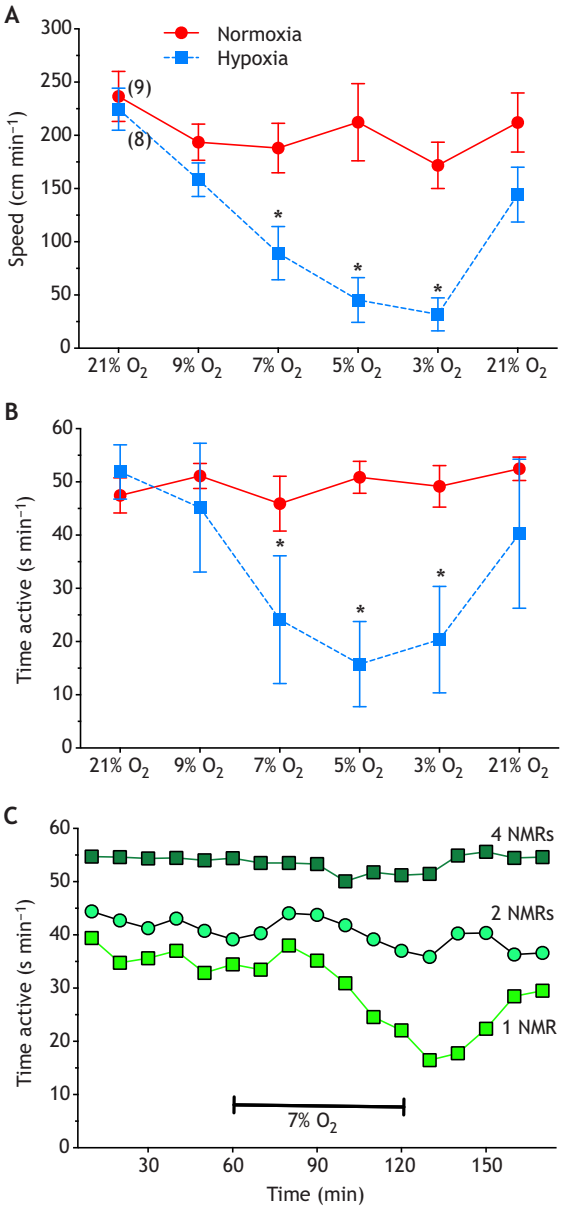


**Fig. 2. NMRs decrease activity in hypoxia but remain awake and active.** Summary of NMR movement speed (A) and time spent active (B) during normoxia or progressively deeper hypoxia. Animals were placed in a large, interconnected, two-chamber enclosure held at 30°C and behaviour was measured using video tracking software as animals were exposed to either 6 h of normoxia (21% $O_2$) or progressively deeper hypoxia followed by reoxygenation (21%, 9%, 7%, 5%, 3% and 21% $O_2$ for 1 h each). Numbers in parentheses indicate number of animals (*n*). Data are means±s.e.m. Asterisks indicate significant differences between normoxia and hypoxia ($P$<0.05). (C) Time spent active for NMRs exposed to 1 h of acute hypoxia (black line), followed by reoxygenation in groups of 1, 2 or 4 animals. Data are means. Adapted from Houlahan et al. (2018) and Ilacqua et al. (2017).

### Thermoregulatory adaptations to hypoxia

In hypoxia, small rodents that can afford to do so reduce their physical activity, seek colder environments and/or reduce huddling behaviour to reduce $T_b$ (i.e. anapyrexia, see Glossary) (Steiner et al., 2002), and conserve energy (Mortola and Feher, 1998; Tattersall and Milsom, 2003). Indeed, given the high costs of thermoregulation for most small mammals, metabolic rate is inherently linked to thermogenesis and moving to a cooler environment typically reduces oxygen demand (Thoresen and Wyatt, 1997; Wood and Gonzales, 1996), provided that this behavioural adaptation is accompanied by a reduction in the $T_b$ set-point (Tattersall and Milsom, 2003). Small rodents such as rats, mice, hamsters and chipmunks exhibit such behavioural thermoregulation when exposed to acute hypoxia (Gordon and Fogelson, 1991; Levesque and Tattersall, 2009), as do a variety of other species, including goldfish, lizards and toads (Wood and Gonzales, 1996).

Comparatively, some mammals, such as high-altitude species, are seasonally exposed to low temperatures in addition to sustained hypobaric hypoxia (McClelland and Scott, 2019). Cold temperatures present a paradox to animals that must stay active and awake in a hypoxic environment: thermoregulation is energetically expensive, but metabolic rate suppression is among the most effective adaptations to hypoxia in small mammals. This conflict is particularly vexing in small rodents that have inherently low thermal inertia as a result of their small body size (Sumbera, 2019). By contrast, NMR burrows are relatively warm (ranging from ~23 to 49°C across seasons and burrow depths; Sumbera, 2019).

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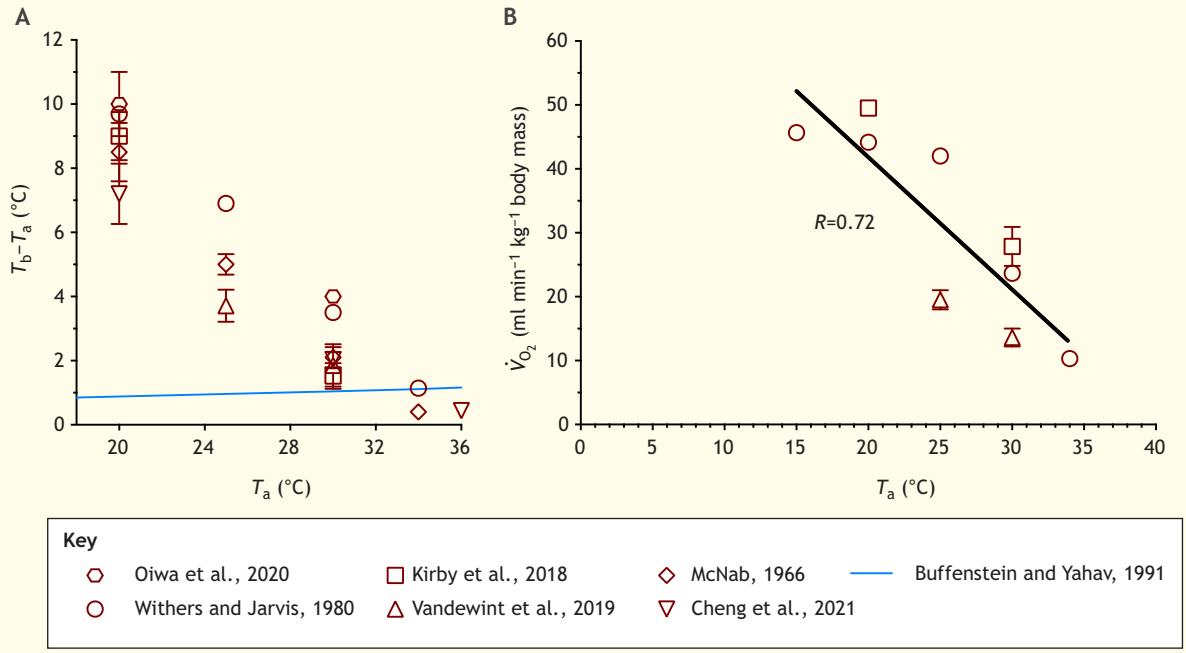
Given this, NMRs have a limited scope for thermogenesis-related energy savings in hypoxia. However, these animals do take advantage of the minimal scope that is available to them. Fortuitously, this enhances the utility of NMRs as a model system for the study of hypoxia tolerance: by removing a confounding environmental variable (thermogenesis in cold climates) that has driven balanced adaptations in many other well-studied hypoxia-tolerant species.

As with behavioural responses, thermoregulatory responses to hypoxia of NMRs have only received attention recently. With regard to behavioural thermoregulation, NMRs do not employ antipyretic strategies, unlike most small mammals in hypoxia (Barros et al., 2004; Clark and Fewell, 1996; Gordon, 1997; Gordon and Fogelson, 1991; Hayden and Lindberg, 1970; Steiner et al., 2002; Tattersall et al., 2002). Specifically, NMRs do not move to colder environments when oxygen is reduced (Kirby et al., 2018), and also do not reduce huddling behaviour (Houlahan et al., 2018). Taken together, these results indicate that NMRs gain energy savings benefits from reduced activity in hypoxia but that behavioural strategies of anapyrexia are not critical to the hypoxia tolerance of this species.

When exposed to acute hypoxia, the $T_b$ of NMRs rapidly drops to within 1°C of ambient temperature ($T_a$) (Cheng et al., 2021b; Ilacqua et al., 2017; Kirby et al., 2018; Pamenter et al., 2019), indicating that thermogenesis is switched off in hypoxia, presumably to conserve energy and reduce metabolic demand (Fig. 1B, see also Box 2). This cooling occurs within minutes of the onset of hypoxia and appears to be the result of shutting off active heating processes as opposed to active heat dissipation. In support of this, inhibiting radiative and evaporative cooling strategies does not alter the hypoxic change in $T_b$ (Vandewint et al., 2019), whereas heat generation from the intrascapular brown adipose tissue (BAT) region of normoxic NMRs is completely absent following <1 h in 7% $O_2$ (Fig. 1C,D; Cheng et al., 2021b), indicating that non-shivering thermogenesis (see Glossary) is actively turned off with the onset of hypoxia. This change is mediated by a rapid decrease in the expression of the thermogenic protein uncoupling protein 1 (UCP1) in intrascapular BAT mitochondria, potentially via a

**Box 2. Thermoregulation in heterothermic naked mole-rats**




Naked mole-rats have among the poorest capacity for thermoregulation of any known adult mammal as a result of various anatomical factors, including a lack of insulating fur, loosely folded and porous skin, and a limited amount of subcutaneous fat. Therefore, naked mole-rats lack sufficient insulation to limit heat transfer across their skin, and are unable to effectively maintain body temperature ($T_b$) endogenously when housed alone and below their thermoneutral zone (Buffenstein and Yahav, 1991; Daly et al., 1997; McNab, 1966; Withers and Jarvis, 1980; Yahav and Buffenstein, 1991a,b). As a result, the $T_b$ of naked mole-rats in isolation is usually just a few degrees above the ambient temperature ($T_a$). Consistent with this, previous experiments in which naked mole-rats were subjected to a high rate of air flow (and therefore enhanced heat loss due to radiative cooling) suggested that the $T_b$ of naked mole-rats conforms very closely with $T_a$; as a result, these animals were labelled as poikilotherms (Buffenstein and Yahav, 1991). However, such experimental conditions are not ecologically relevant to this species and numerous studies have demonstrated that in lower air flow conditions and in temperatures below thermoneutrality, naked mole-rats maintain $T_b$ well above $T_a$ (A) and their metabolic rate increases substantially with decreasing $T_a$ (B), suggesting increased energy expenditure on thermoregulation (Cheng et al., 2021b; Kirby et al., 2018; McNab, 1966; Oiwa et al., 2020; Vandewint et al., 2019; Withers and Jarvis, 1980). These findings are summarized in the figure, showing the difference between $T_b$ and $T_a$ in experimental temperatures at and below the thermoneutral zone of this species. The predicted $T_b$–$T_a$ differential from Buffenstein and Yahav (1991) is described by the blue line. Interestingly, huddling or the provision of insulation further reduces the metabolic rate of naked mole-rats in the cold and ameliorates cold-mediated reductions in $T_b$. Taken together, these studies demonstrate that, like other small mammals, naked mole-rats attempt to thermoregulate via non-shivering thermogenesis (see Glossary) in cold environmental temperatures and employ behavioural and environmental means in support of this endogenous heat generation. As such, they are accurately described as heterotherms (see Glossary) and are not poikilothermic.

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mitochondrial fission mechanism. Given the NMRs' lack of insulation, the high degree of heat generated via non-shivering thermogenesis in normoxia (combined with the continuous loss of this heat at temperatures below thermoneutrality) likely contributes significantly to energy demand (Kirby et al., 2018; McNab, 1966; Withers and Jarvis, 1980). Thus, shutting down this energy drain in hypoxia may confer significant energy savings.

Changes in $T_b$ with acute hypoxic exposure have also been examined in numerous other species of African mole-rats, and many of these species are capable of robust metabolic rate suppression in hypoxia; however, the magnitude of the decreases in metabolic rate and $T_b$ in all other mole-rat species examined is less than in NMRs (Devereaux and Pamenter, 2020; Ivy et al., 2020; Zhang and Pamenter, 2019). Intriguingly, each of these species also exhibits decreases in $T_b$ with acute hypoxia and, in many cases, $T_b$ is reduced to near $T_a$. Other African mole-rats, like most mammals that are fossorial (see Glossary), have a resting $T_b$ of ∼34–36°C, and live in relatively warm and stable environmental temperatures ranging from ∼12 to 36°C, depending on seasonality and elevation (Sumbera, 2019); thus, they can potentially take advantage of a greater thermal scope in hypoxia than can NMRs. However, the cost of thermoregulation in NMRs is likely much higher than in furry African mole-rats, and so although the absolute change in $T_b$ with acute hypoxia is smaller in NMRs, the energy savings are likely greater, thus contributing to the more robust hypoxic metabolic suppression in this species.

### Cellular adaptations to hypoxia – decreasing demand

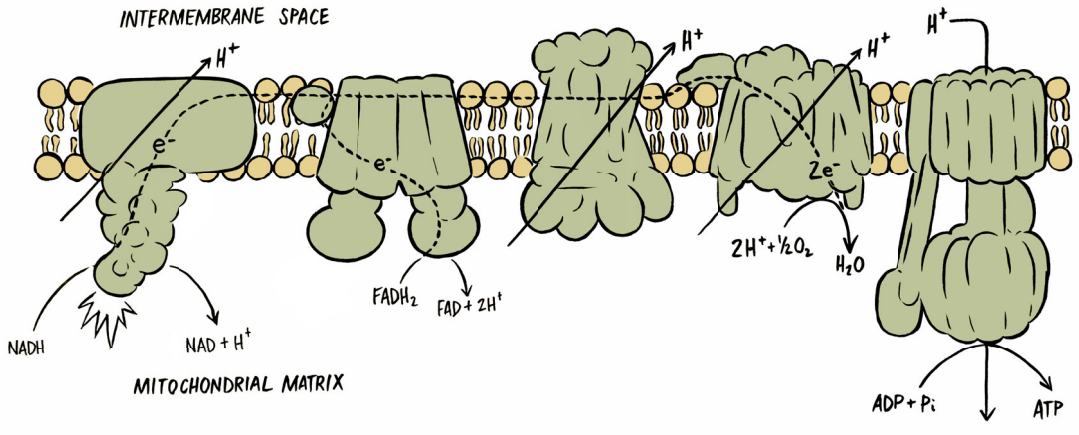
Behavioural and physiological strategies of metabolic rate suppression provide robust energy savings in hypoxia, but energy savings at the cellular level achieved by increasing metabolic efficiency or reducing ATP-demanding cellular functions also play a critical role in hypoxia tolerance. Indeed, many studies have demonstrated alterations in metabolic pathways or temporary cessation or reductions of non-essential cellular function during hypoxia. These include changes in mitochondrial function, and reductions in gene transcription/translation, protein synthesis and ion channel permeability and expression, among others (Bickler and Buck, 2007; Boutilier, 2001; Hochachka, 1986; Hochachka et al., 1996).

### Mitochondrial plasticity in hypoxia

Changes in mitochondrial metabolism and function during hypoxia can support (or reflect) energy savings and also improve the efficiency of the generation of cellular energetic substrates (e.g. ATP). In particular, there are numerous examples of plasticity in mitochondrial expression, function and signalling following acclimation to low oxygen in various hypoxia-tolerant vertebrates (Devaux et al., 2019; Galli and Richards, 2014; Hickey et al., 2012; Pamenter, 2014). For example, following 2 weeks of anoxic exposure, brain tissue from anoxia-tolerant red-eared slider turtles (*Trachemys scripta*) has reduced mitochondrial density, and mitochondria exhibit reduced flux through the electron transport chain (ETC; see Box 3) and a reduction in $F_1F_O$-ATPase activity (Pamenter et al., 2016). Similar reductions in $F_1F_O$-ATPase activity following weeks of anoxic acclimation occur in heart muscle from red-eared slider turtles (Galli et al., 2013), and skeletal muscle from the anoxia-tolerant common frog (*Rana temporalis*), where the phenomenon was first described in a seminal paper (St-Pierre et al., 2000). This adaptation is associated with prevention of ATP consumption due to reversal of the $F_1F_O$-ATPase during anoxia.

Few studies have examined mitochondrial plasticity following acclimation to hypoxia in hypoxia-tolerant mammals; however, in

**Box 3. Mitochondrial energetics**




Mitochondria are the linchpin of aerobic cellular metabolism. In normoxia, mitochondria consume >90% of inspired oxygen to facilitate the pumping of protons ($H^+$) across the inner mitochondrial membrane by the electron transport chain (ETC; Rolfe and Brown, 1997). Specifically, NADH and FADH, which are generated in the Krebs cycle, are oxidized by complexes I and II of the ETC, respectively. This releases electrons and protons, with the former being passed along the ETC towards complex IV (cytochrome oxidase), and the latter being pumped across the inner mitochondrial membrane. This generates the proton-motive force that energizes the phosphorylation of ADP to ATP by the $F_1F_O$-ATPase (complex V). Through this process of oxidative phosphorylation, mitochondria generate the majority of a cell's energy (Ainscow and Brand, 1999; Benard et al., 2006; Hinkle, 2005; Huttemann et al., 2007; Mitchell and Moyle, 1967; Pamenter, 2014). This is an oxygen-dependent process because of the final reaction in complex IV, where oxygen and protons combine to form $H_2O$. During periods of limited oxygen availability, this final step in the ETC is impaired, and oxidative phosphorylation is constrained or abolished, leading to energy deficits at the cellular level and an increased reliance on anaerobic energy production.

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high-altitude populations of deer-mice acclimated to hypoxia for 6–10 weeks, skeletal muscle mitochondria exhibit reduced flux through complex IV of the ETC, higher respiratory control ratios (indicative of more tightly coupled and therefore 'healthy' mitochondria) and reduced $H_2O_2$ generation (Mahalingam et al., 2017). These adaptations likely provide improved mitochondrial efficiency to maximize skeletal muscle energy production with reduced oxygen availability.

In NMRs, adaptations at the mitochondrial level in intrascapular BAT, skeletal and cardiac muscle, and brain hint at improved mitochondrial energetics and/or downregulation of mitochondrial function during hypoxia. In BAT, the expression of mitochondrial ETC complexes I–V and UCP1 is downregulated by 30–70% within 1 h of acute *in vivo* hypoxia (Cheng et al., 2021b). UCP1-mediated mitochondrial uncoupling in BAT is the primary mechanism of cold-induced thermogenesis in small mammals (i.e. non-shivering thermogenesis) and this reduction in UCP1 expression likely explains the rapid reduction in heat generation in hypoxic NMRs (see above). Intriguingly, similar decreases in UCP1 expression are observed in three other social African mole-rat species, but not a solitary mole-rat species (Cheng et al., 2021b), suggesting that this mechanism has evolved preferentially with sociality in this lineage.

Conversely, in skeletal muscle, exposure to acute (4 h in 7% $O_2$) or chronic hypoxia (4–6 weeks in 11% $O_2$) results in mild (~15%) reductions in coupled and uncoupled mitochondrial respiration (Cheng et al., 2021a). In heart, NMR cardiac mitochondria have lower complex II respiration rates and respiration is more tightly coupled to the proton gradient than in mice (Lau et al., 2020), suggesting enhanced respiration efficiency. Finally, in brain, mitochondrial respiration is more tightly coupled to the proton gradient after 4 h of 3% oxygen than in control animals (Pamenter et al., 2018), indicating that mitochondrial energy production is more efficient in this tissue following hypoxic exposure (Fig. 3). In addition, total ETC flux is downregulated by ~85% after acute hypoxia, which is similar to the suppression of whole-animal metabolic rate under these conditions. This variation in the scope and magnitude of mitochondrial adaptations to hypoxia between tissues is fascinating as it suggests not only variable sensitivity to hypoxia within different tissues but also a varied demand for sustained function of each of these tissues in hypoxia, which may have driven unique adaptations across different organs within a single species.

## Alterations of transcription and protein synthesis

Transcription and protein synthesis are energetically expensive, and these functions are rapidly downregulated with the onset of hypoxia to conserve energy in both hypoxia-tolerant and -intolerant species (Hochachka, 1986). Interestingly, following *in vivo* hypoxia (4 h in 7% $O_2$), micro-RNA (miRNA) changes are consistent with the suppression of protein synthesis via numerous pathways in NMR brain (Hadj-Moussa et al., 2021b). Specifically, hypoxia-responsive miRNAs target transcription of small and large ribosomal proteins and transcription factors in a manner that is consistent with inhibiting these processes. In addition, protein biosynthesis is directly inhibited in NMR brain, muscle and lung via modulation of the mTOR/Akt pathway during hypoxia (Al-Attar et al., 2020).

Conversely, some genes and proteins – such as hypoxia-inducible factor (HIF) and its downstream targets – are upregulated by hypoxia in most species and activate cellular responses (both deleterious and cytoprotective; Liu and Simon, 2004). Intriguingly, advances in genome analysis across hypoxia-tolerant animal and human populations indicate that the most commonly reported adaptation-associated genes (those mutated by evolutionary pressures associated with life in low oxygen) are related to the control and function of the HIF pathway and/or downstream genes (Pamenter et al., 2020). These mutations commonly result in chronic upregulation of HIF function; therefore, these genomic findings suggest that such species likely express endogenously high levels of HIF and downstream transcriptional targets. This is true for NMRs, which express a mutation in the binding domain of von Hippel–Lindau protein (Kim et al., 2011), a protein which normally tags HIF for degradation by the proteasome (Haase, 2009). As a result, HIF-1α protein expression is higher in NMRs than in mice under normoxic conditions, and its expression is also more robustly increased during hypoxia (Pamenter, 2017; Xiao et al., 2017). Furthermore, downstream cytoprotective and anti-apoptotic proteins regulated by HIF are upregulated in NMR brain during *in vivo* hypoxia (4 h in 7% $O_2$; Hawkins et al., 2019). Conversely, upregulation of HIF can lead to activation of pro-death pathways following hypoxia or chemical anoxia in mouse brain (Xu et al., 2011; Zhang et al., 2007).

## Channel arrest in brain

In electrically active tissues (brain and heart), cellular energy is primarily consumed by the activity of ATPases to maintain ion

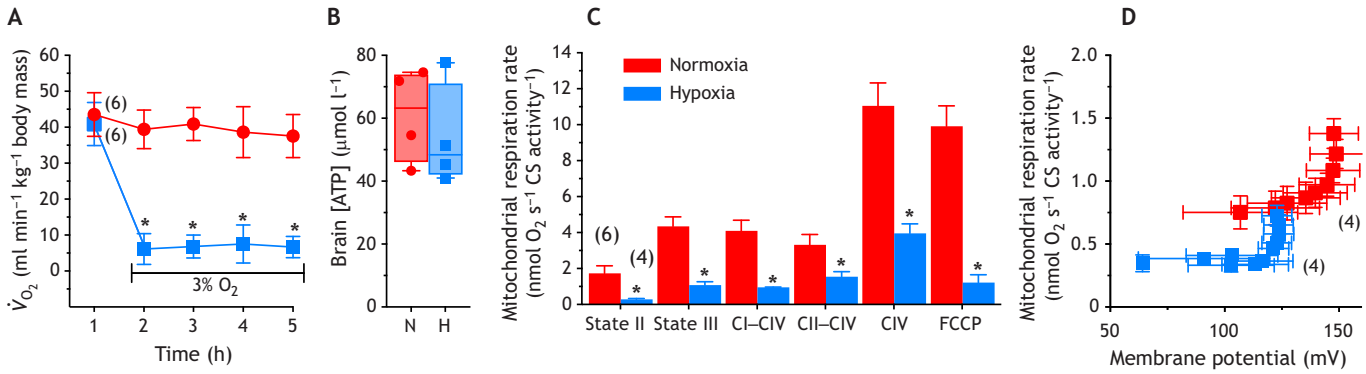


**Fig. 3. NMR brain mitochondrial function is reduced following *in vivo* hypoxia.** (A) Adult subordinate NMR whole-animal $O_2$ consumption rates in normoxia (21% $O_2$, red) or hypoxia (3% $O_2$, blue). (B) Brain [ATP] following these treatments. (C) Comparison of complex I- and II-fuelled, and FCCP-uncoupled brain mitochondrial respiration rates (note: FCCP is a protonophore that permits $H^+$ to cross the mitochondrial membrane and thus uncouples oxidative phosphorylation from the electron transport cycle). (D) Hypoxic brain mitochondrial $H^+$ flux and $O_2$ consumption are equally coupled but at a lower membrane potential relative to controls. Data are means±s.e.m. *n* is given in parentheses . Asterisks indicate significant difference from normoxic controls ($P$<0.05). Adapted from Pamenter et al. (2019, 2018).

gradients across the cellular and organelle membranes (Erecinska and Silver, 1989). The $Na^+/K^+$-ATPase alone is estimated to use ∼50% of the ATP budget in rodent brain (Engl and Attwell, 2015). Thus, to support metabolic rate suppression at the cellular level, it is beneficial to decrease ATPase activity. For example, in anoxia-tolerant *T. scripta* brain, $Na^+/K^+$-ATPase activity reversibly decreases by ∼31–35% following 24 h of anoxia (Hylland et al., 1997), and $Na^+/K^+$-ATPase subunit expression decreases ∼50% following weeks of anoxic conditions at 3°C (Stecyk et al., 2017). In NMR brain, $Na^+/K^+$-ATPase activity decreases following 4 h of *in vivo* hypoxia in a region-specific manner that likely supports maintenance of autonomic function and increased activity in brain regions associated with exploration and navigation, while reducing overall brain energy demand primarily due to a robust $Na^+/K^+$-ATPase activity reduction in forebrain (Farhat et al., 2021). With longer-term hypoxia (11% $O_2$ for 4–6 weeks), whole-brain $Na^+/K^+$-ATPase activity decreases ∼75% (Farhat et al., 2020).

A reduction in ion pumping without a concomitant reduction in ion leak across membranes results in the rundown of cellular ion gradients. Given this, Hochachka (1986) suggested that arrest of ion channels must occur in the brains of hypoxia-tolerant species (Hochachka, 1986). Specifically, the channel arrest hypothesis predicts that a hypoxia-tolerant brain will have (1) a lower basal expression of synaptic proteins, and (2) the ability to downregulate the function of those proteins to conserve energy during periods of low oxygen stress. Channel arrest of excitatory glutamatergic receptors has so far been demonstrated in the brains of anoxia-tolerant freshwater turtles and goldfish (Bickler et al., 2000; Buck and Bickler, 1995; Pamenter et al., 2008a,b; Wilkie et al., 2008).

Although the channel arrest hypothesis has not been directly tested in any mammalian brain, it is fascinating to note that initial studies support this hypothesis in NMRs. For example, glutamate concentration decreases in NMR brain following 4 h of *in vivo* hypoxia (Cheng et al., 2022), whereas increased glutamate is a hallmark of excitotoxic cell death in hypoxia-intolerant mammal brain during periods of hypoxia or ischaemia (see Glossary; Rossi et al., 2000). Furthermore, NMR *N*-methyl-D-aspartate receptors (NMDARs) are largely composed of NR2D subunits (Peterson et al., 2012b). This subunit type is commonly expressed in neonatal mammals and has a lower conductance to excitatory $Ca^{2+}$ molecules than do NMDARs in most adult mammals, which are typically composed of higher conductance subunits (Law et al., 2003). Rapid $Ca^{2+}$ entry is a key component of excitotoxic brain cell death in hypoxia and ischaemia (Choi, 1992; Choi and Rothman, 1990), and thus this receptor architecture is consistent with low-throughput $Ca^{2+}$ influx characteristic of the hypoxia-tolerant neonatal brain. Indeed, NMR hippocampal slices exhibit a blunted $Ca^{2+}$ influx response following hypoxia or $K^+$ perfusion relative to both adult and neonatal rat brain slices (Peterson et al., 2012a). Clearly, further experiments are warranted to better understand the modulation of glutamatergic signalling, and of cellular ion flux in general, in this model of hypoxia tolerance.

## Achieving metabolic balance – increasing supply

### Increased flux through the oxygen transport cascade

When most adult mammals encounter hypoxia, their first physiological reflex is typically to hyperventilate to increase the supply of oxygen to the lungs. This is termed the 'hypoxic ventilatory response' (see Glossary), and it is a reflex increase in breathing that occurs rapidly in response to the detection of decreased oxygen availability by the mammalian chemoreceptors (Pamenter and Powell, 2016; Prabhakar, 2000). Importantly, there are numerous possible combinations of changes to ventilation and metabolism, and, as a result, the hypoxic ventilatory response is quite variable in mammals (Frappell et al., 1992). In broad generalities, most adult mammals respond to hypoxia with an increase in ventilation (i.e. the traditional hypoxic ventilatory response), and/or small decreases in metabolic rate and $T_b$ set-point (Frappell et al., 1992; Pamenter and Powell, 2016), although this is by no means a rule.

As mentioned above, increasing ventilation to compensate for reduced oxygen content in the inspired air is energetically expensive and is therefore a costly long-term strategy for tolerating hypoxia. Given this, it is not surprising that, in addition to robust hypoxic metabolic responses (see previous section), many hypoxia-tolerant mammals also exhibit a blunted hypoxic ventilatory response, which manifests either as a smaller increase in ventilation relative to that of hypoxia-intolerant mammals or a response that is initiated at lower inspired oxygen levels (Arieli and Ar, 1979; Boggs and Birchard, 1989; Boggs et al., 1984). For example, the blind mole-rat (*Spalax ehrenbergi*) does not mount a ventilatory response to hypoxia above 8% $O_2$ (Arieli and Ar, 1979), whereas rats and humans mount ventilatory responses to 14–16% inspired $O_2$ (Boggs et al., 1984; Frappell et al., 1992), as do hypoxia-intolerant subterranean rodents (Devereaux et al., 2021). In addition, hypoxia-tolerant mammals often alter their breathing strategy in acute hypoxia by increasing tidal volume (see Glossary), while concomitantly decreasing breathing frequency (Boggs et al., 1984). Increasing tidal volume is thought to maximize the efficiency of the respiratory system in hypoxia by reducing dead-space ventilation (see Glossary; Tenney and Boggs, 2010), which presumably outstrips the metabolic cost of increasing tidal volume (Vitalis and Milsom, 1986).

Unique among mammals studied to date, NMRs do not appear to upregulate ventilation or heart rate in hypoxia. Specifically, they do not exhibit a traditional hypoxic ventilatory response (Dzal et al., 2019; although they may do so in deeper levels of hypoxia than those examined to date), and heart rate decreases with acute hypoxia (Pamenter et al., 2019; albeit not to the same degree as overall metabolic rate, suggesting an increase in cardiac output relative to systemic metabolic demand with progressive hypoxia). An important caveat is that ventilatory responses of NMRs to hypoxia have to date only been measured using the plethysmography technique, which relies on the temperature differential between $T_b$ and $T_a$ to detect breaths non-invasively from freely behaving animals. NMRs have a small temperature differential in normoxia but this gradient is largely abolished in hypoxia (see above); therefore, the accuracy of plethysmography measurements of tidal volume in hypoxic NMRs is questionable. Breathing frequency does decrease significantly in hypoxic NMRs (Dzal et al., 2019; Pamenter et al., 2015, 2019), but measurements of tidal volume in hypoxia using pneumotachography are needed to confirm the absence of a hypoxic ventilatory response in this species. Finally, beyond ventilation and perfusion, the diffusion barrier between the lungs and blood is, if anything, greater in NMRs than in hypoxia-intolerant rodents (Maina et al., 2001). Thus, taken together, these findings suggest that, other than blood oxygen-binding characteristics, NMRs do not exhibit adaptations within the oxygen transport cascade (see Glossary and Box 4) that increase oxygen supply to tissues during hypoxia, as is commonly observed in most other adult mammals.

Given their remarkable hypoxia tolerance, it is possible that the unique adaptive response to acute hypoxia in NMRs (i.e. robust metabolic rate depression with apparently negligible changes in

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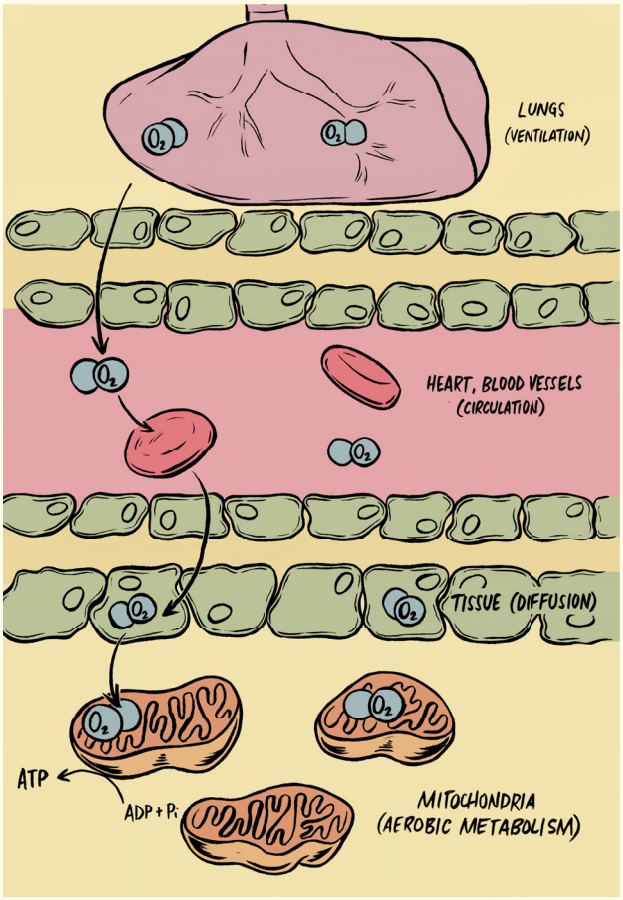
**Box 4. The oxygen transport cascade**




Complex multi-cellular organisms require integrated physiological systems to deliver environmental oxygen to cells, where it is ultimately consumed by mitochondria in the production of the primary cellular energy substrate: ATP (see Box 3). This series of physiological steps is termed the oxygen transport cascade (see Glossary). This cascade is composed of four primary steps: ventilation at the lungs (breathing), diffusion of oxygen from the air into the blood across the lung surface area, circulation (oxygen molecules bind to haemoglobin in red blood cells, which are pumped around the body by the activity of the heart), and diffusion of oxygen from the blood into the cells. At sea level, oxygen makes up ∼21% of inspired air (∼160 Torr), but at each step of the oxygen transport cascade this percentage is reduced, such that at the cellular level the oxygen tension may be as low as ∼5 Torr (di Prampero, 1985; Weibel, 1984). Maintaining this gradient is essential to the function of the oxygen transport cascade because the movement of oxygen into the blood, and from the blood into the tissues, occurs via passive diffusion.

ventilation) represents an extreme on the spectrum of physiological responses to low oxygen. We can begin to understand the evolutionary origin of these adaptations by studying similar abilities in related species and in other hypoxia-tolerant mammals. For example, it is possible that animals that are evolutionarily related to NMRs, and that live in similar social groupings and/or environments, will express a similarly high tolerance to hypoxia and a similar adaptive phenotype in their physiological response to hypoxia. To test this hypothesis, we recently measured physiological responses to hypoxia in 11 cousin species of African mole-rats, including Damaraland mole-rats, which are the only other eusocial mammals (Devereaux and Pamenter, 2020; Ivy et al., 2020; Zhang and Pamenter, 2019). Remarkably, we found that almost all species of mole-rats are capable of withstanding severe low-oxygen challenges, with most species tolerating hypoxia as low as 2–3% $O_2$ for 30 min before exhibiting signs of distress or losing consciousness. Surprisingly, however, these species display a range of ventilatory and metabolic responses to hypoxia, none of which closely resembles that of NMRs. As predicted, most other African mole-rats exhibit a strong metabolic rate suppression with progressive hypoxia; however, the magnitude of this response is considerably smaller than in NMRs, and all other African mole-rat species also increase ventilation in hypoxia. The onset of ventilatory responses is, however, blunted in these species relative to hypoxia-intolerant rodents, and also tends to favour increases in tidal volume over breathing frequency in severe hypoxia, consistent with previous studies in hypoxia-tolerant rodents (Boggs and Birchard, 1989; Boggs et al., 1984; Ivy and Scott, 2017).

### Upregulating glycolysis?

Whereas most hypoxia-tolerant species exhibit robust metabolic rate suppression in hypoxia, hypoxia-intolerant species typically attempt to upregulate anaerobic metabolism to maintain ATP supply (Seagroves et al., 2001). However, this strategy is usually maladaptive because of the limited systemic glycogen supply to fuel glycolysis and progressive cellular acidification resulting from ATP hydrolysis (Robergs et al., 2004), and/or the accumulation of acidic metabolic end-products (primarily lactic acid; Webster et al., 1999). Beyond the retarding impact of hypoxia on oxidative phosphorylation, cellular acidification also has significant impacts on aerobic mitochondrial respiration because deviations from physiological pH (acidic or alkaline) may shift the mitochondrial proton gradient, alter the kinetics of mitochondrial ETC enzymes, impact reactive oxygen species (ROS) generation, or damage mitochondrial components, among other effects (Genders et al., 2019; Holtzman et al., 1987; Santo-Domingo and Demaurex, 2012; Selivanov et al., 2008; Wilson and Forman, 1982). Thus, unbuffered changes in pH may further impair aerobic ATP production in hypoxia and may also promote cell death.

Nonetheless, many hypoxia-tolerant species rely on glycolytic throughput to meet their drastically reduced energy demands in severe hypoxia. And of course, in anoxia, anaerobic metabolism is the only option available. In support of this necessity, anoxia-tolerant species have developed elegant strategies to moderate the impact of metabolic acidosis arising from a sustained reliance on anaerobic metabolism. For example, some species produce alternative end-products that help to reduce or prevent backlogs of metabolic pathways and cellular acidification, while other species have robust pH-buffering capacities. Perhaps the most famous example of the former is the ability of crucian carp (*Carassius carassius*) to utilize ethanol as an alternative end-product to lactate during anoxia (Shoubridge and Hochachka, 1980), thereby avoiding (or at least reducing) metabolic acidosis. Conversely, anoxia-tolerant freshwater turtles sequester protons into their bones and shell, and thereby buffer cellular acidification (Jackson et al., 2000; Reese et al., 2004). NMRs appear to also have a robust capability to buffer cellular acidification: inhalation of <10% $CO_2$ does not result in cellular acidification, whereas mice experience systemic acidification with inhaled $CO_2$ as low as 1% (Park et al., 2017). Although the mechanism underlying this buffering capacity is unknown, these *in vivo* data hint at adaptations that could

mitigate pH shifts arising from increased anaerobic metabolism in hypoxia.

The degree to which NMRs rely on glycolysis when oxygen is limited is an open-ended question and likely depends on the severity and duration of hypoxic stress experienced. Indirect *in vivo* evidence obtained using whole-animal respirometry demonstrates that NMRs undergo a sustained metabolic fuel switch from primarily lipids to entirely carbohydrate metabolism when in hypoxia (Pamenter et al., 2019). In addition, liver glucose stores are mobilized and blood glucose concentration rises during hypoxia, which may support an increased reliance on carbohydrate metabolism, or may instead reflect decreased use of this substrate by other organs. [Note that although lipids contain more ATP $g^{-1}$ than do carbohydrates, the ATP yield per mole of $O_2$ catabolized is 15–30% higher when derived from carbohydrates than from lipids (as a result of the higher energetic costs of breaking high-energy bonds in lipids) and thus a greater reliance on carbohydrates increases energetic efficiency during acute hypoxia (Brand, 2005; Hochachka, 1985).] This strategy would be useful in NMRs because they likely experience hypoxia only transiently during intense exercise (e.g. when digging tunnels) or when sleeping, and thus would have opportunities to replenish carbohydrate stores while in regions of their burrows with higher levels of oxygen, when they rely more heavily on lipid energy stores (Dzal et al., 2019; Pamenter et al., 2019).

There are two outstanding questions in this area: which organs are using glucose mobilized from the liver?; and what mechanism mediates a putatively organ-specific reorganization of metabolic fuel use in hypoxia? NMRs remain physically active in hypoxia, and thus it would seem intuitive that NMR muscle makes use of this fuel source under hypoxic conditions. However, in skeletal muscle from NMRs exposed to 4 h of severe hypoxia (3% $O_2$), glutamate transporter 4 (GLUT4, the primary mediator of glucose uptake into cells), the key glycolytic enzyme glycogen synthase, and phosphorylation of AMP kinase (AMPK: the upregulation of which typically mediates increased glycolysis; Hardie et al., 2012), all decrease (Hadj-Moussa et al., 2021a), consistent with decreased glycolytic throughput in this tissue. Similarly in liver, lactate concentration decreases following 4 h of 7% *in vivo* hypoxia (Cheng et al., 2022). In contrast, in brain, and following a similar acute *in vivo* hypoxic exposure of 7% $O_2$ for 4 h, lactate concentration increases (Cheng et al., 2022), as does lactate dehydrogenase (LDH) protein expression, whereas the expression of SREP2, a key regulator of fatty acid synthesis, decreases (Hadj-Moussa et al., 2021b). These changes suggest the occurrence of increased glycolytic throughput in this tissue. Consistent with these organ-specific observations, tissue acidity, which typically occurs with increased glycolytic metabolism, is observed in brain but not liver, muscle or heart following *in vivo* hypoxia (Pamenter et al., 2019).

Whereas metabolic suppression may be organ specific in acute hypoxia, NMRs appear to suppress metabolic systems broadly with longer-term hypoxia of weeks to months. Specifically, the activities of key enzymes of glycolysis [pyruvate kinase (PK) and LDH], the TCA cycle (citric synthase) and fatty-acid oxidation (carnitine palmitoyl transferase and 3-hydroxyacyl-CoA dehydrogenase), all decrease markedly in muscle and liver after 4–6 weeks of *in vivo* hypoxia (11% $O_2$) but remain largely unchanged in heart and brain (except for PK, which is decreased in both) (Farhat et al., 2020).

These tissue-specific changes hint at regional control in energetic pathways such that energy pools are prioritized in hypoxia for brain and possibly heart, both of which are more sensitive to oxygen deficits and whose function is requisite for survival. Further support for this divergent metabolic response comes from a recent study showing that, relative to C57/BL5 mice, NMRs have very high levels of glycogen stores in heart (Faulkes et al., 2019), suggesting this organ is primed for sustained anaerobic function. Taken together, these data support the likelihood that glycolysis is a key contributor to the energy metabolism of at least some tissues in hypoxic NMRs, but further work is required in this area.

#### Fructolysis: an option only in anoxia?

A recent paper demonstrated that, in anoxia, NMRs mobilize both glucose and fructose *in vivo*, and that NMR brain and heart are able to metabolize fructose *in vitro* (Park et al., 2017). This would allow NMR tissues to circumvent phospho-fructokinase-1 (PFK), which is a key checkpoint regulator of glycolysis that is normally inhibited by a variety of cellular factors, including acidic pH levels and hypoxia (Bartrons and Caro, 2007). Therefore, an ability to utilize fructose in anoxia would allow NMRs to continue using glycolysis to supply ATP under anoxic conditions. However, the anoxia-induced increase of blood glucose concentration in NMRs *in vivo* is considerably greater than that of fructose, and the source of metabolic fructose in NMRs is unclear.

So, what is the primary carbohydrate fuel source in hypoxic NMRs? An examination of metabolic pathways in NMR brain following 4 h of acute *in vivo* hypoxia (7% $O_2$) reveals a hypoxia-mediated downregulation in protein expression of the fructose transporter GLUT5, and also in ketohexokinase, which is the enzyme responsible for catalysing the first step of fructose catabolism (Hadj-Moussa et al., 2021b). These data suggest that fructose metabolism is inhibited in NMR brain during ecophysiologically relevant hypoxic exposures. Importantly, fructose metabolism also occurs in pathophysiological heart disease in humans (Mirtschink et al., 2015), and it is possible that the ability of NMRs to mobilize and metabolize fructose in acute anoxia reflects a ‘last-ditch’ effort to survive in such harsh environmental conditions. If this were the case, we would not expect NMRs to mobilize and metabolize fructose at sublethal levels of hypoxia. Thus, this fascinating metabolic trick may be limited to anoxic conditions, but further studies are warranted to evaluate the relative roles of fructose and glucose in fuelling glycolytic metabolism in ecophysiologically relevant levels of hypoxia.

### Cytoprotective strategies in hypoxia

For most hypoxia-tolerant species, downregulating metabolism is only a part of a complex suite of cellular adaptations to low-oxygen stress. Another major pillar of defence is the upregulation of cellular mechanisms that activate cytoprotective pathways, inhibit cell death pathways, and mitigate damage to biomolecules. For example, in NMR brain, several neuroprotective pathways are upregulated by acute hypoxia, including HIF-1a, which drives changes in numerous protective markers, including the enzymes CA9 (which balances cellular pH) and CITED2 (which inhibits proapoptotic genes and activates pro-survival genes; Hawkins et al., 2019). Another key pathway upregulated by hypoxia in NMR brain is the redox-sensitive NFκB pathway (which decreases apoptosis, activates antioxidant defences, and regulates inflammation and DNA damage repair; Hawkins et al., 2019). miRNA changes that are generally consistent with inhibiting cell death pathways are also upregulated following *in vivo* hypoxia in the brains of NMRs and also other African mole-rat species (Hadj-Moussa et al., 2021b; Logan et al., 2020).

#### Redox homeostasis

Another key regulator of cellular signalling pathways, and a major contributor to biomolecular damage during hypoxia and

reoxygenation, are imbalances in free radicals. Indeed, repeated episodes of intermittent hypoxia and reoxygenation are expected to pose a significant redox challenge in mole-rat species if they experience frequent deleterious bursts of ROS and cellular damage associated with rapid reoxygenation following hypoxia (or ischaemia; Chouchani et al., 2014). Interestingly, previous studies have found that NMRs accumulate more oxidized protein, lipid and DNA damage than do mice (Andziak et al., 2006; Perez et al., 2009). However, these studies focused on longevity and compared developmentally matched animals (i.e. 3 month old mice with 2 year old NMRs). When comparisons are made between age-matched animals, these significant changes largely disappear (or the data are not presented), suggesting that the rate of accumulation of oxidative damage is similar between species, and possibly greater in mice. Furthermore, these studies were conducted in laboratory populations that were not exposed to natural cycles of hypoxia–reoxygenation, as NMRs would be in nature (Buffenstein et al., 2022). Presumably such life history exposure to repeated bouts of hypoxia–reoxygenation would increase the accumulation of oxidative damage in both species, and likely more so in the hypoxia-intolerant mouse, in which exposure to repeated intermittent hypoxia is associated with increased redox damage (Shan et al., 2007).

Other studies have compared the expression of antioxidant enzymes between NMRs and mice. An early study in this area examined the expression of various antioxidant systems in the liver of developmentally matched NMRs and mice and concluded that NMRs have lower antioxidant capacity as a result of a near-complete absence of glutathione peroxidase in liver (Andziak et al., 2005). However, NMRs have similar or higher levels of both copper/zinc superoxide dismutase and manganese superoxide dismutase, and catalase, and the levels of these enzymes do not decline with age, as they do in mice. Importantly, the deficiency in glutathione peroxidase appears to be tissue specific, as NMR heart and skeletal muscle each produce approximately the same amount of ROS as matched tissues from mice, but are capable of buffering severalfold more $H_2O_2$ (Munro et al., 2019). The difference in scavenging capacity between species is largely due to considerably greater glutathione peroxidase activity in the NMR tissues.

Finally, NMRs may be more capable of remediating cellular damage resulting from redox insults. The NMR genome has a higher copy number of the DNA repair genes, which protects telomere integrity (MacRae et al., 2015). NMRs also express a highly functional telomeric protein TRF1, the efficacy of which increases in low-oxygen conditions, supporting enhanced telomere protection in hypoxia (Augereau et al., 2021). NMR fibroblasts also have more efficient excision repair mechanisms following a radiation challenge (Evdokimov et al., 2018). Importantly, mechanisms that protect biomolecules against ageing and radiation likely also provide protection against redox stress during hypoxia–reoxygenation (Ruby et al., 2018).

### Proteostasis

NMRs have robust mechanisms for maintaining proteostasis (see Glossary), are more resistant to urea-induced protein unfolding and ubiquitination activity, and have higher proteosomal activity (Perez et al., 2009). However, no studies have directly examined any of these responses in hypoxia. With decreased protein synthesis during hypoxia, proteostasis of previously synthesized proteins becomes increasingly important to the maintenance of protein function, and it is interesting to note that the expression of chaperone heat-shock proteins (HSPs) increases during hypoxic exposure in both tolerant and intolerant species, including mice, rats, rabbits, piglets, flies, nematodes, anoxia-tolerant freshwater turtles and estuarine fishes (Baird et al., 2006; Liu et al., 2006; Mestril et al., 1994; Ramaglia and Buck, 2004; Shen et al., 2005; Tiedke et al., 2014). However, synthesis of HSPs is energetically expensive, and a recent study reveals an elegant solution to this problem in NMRs. Specifically, ATP-dependent HSP70 and HSP90 gene and protein expression are markedly reduced after 24 h in 7% $O_2$, whereas the expression of ATP-independent HSP27 and HSP40 proteins remains generally constant (Nguyen et al., 2019). This likely helps to minimize energy expenditure while maintaining proteostasis during hypoxia.

## The neotenic theory of hypoxia tolerance

A tantalizing commonality shared by hypoxia-intolerant mammals is that they are relatively hypoxia tolerant as neonates. This longstanding observation led to the important hypothesis that the evolution of hypoxia tolerance in mammals derives from the retention of neonatal characteristics into adulthood (Harris et al., 2004). Unfortunately, this hypothesis has not yet been examined in an integrated fashion in any hypoxia-tolerant species.

In light of this hypothesis, it is notable that, even beyond their endogenous neotenic haemoglobin isoforms (Johansen et al., 1976), many physiological responses of NMRs to hypoxia are similar to those of neonatal rodents (Dzal et al., 2020). For example, like NMRs, neonatal mammals respond to hypoxia with a blunted hypoxic ventilatory response, robust metabolic rate suppression and decreased thermogenesis (Mortola, 1999; Mortola et al., 1989). In most adult hypoxia-intolerant mammals, ventilatory and metabolic responses to hypoxia are regulated by excitatory glutamatergic signalling pathways (Pamenter et al., 2014a,c; Pamenter and Powell, 2016), whereas in neonates these are regulated by inhibitory adenosinergic and γ-amino butyric acid (GABA)-ergic pathways (Johansson et al., 2001; Wong-Riley et al., 2019). Intriguingly, the central nervous system signalling pathways that regulate physiological responses to hypoxia in adult NMRs match those of neonatal mammals (Chung et al., 2016; Dzal et al., 2019; Pamenter et al., 2015). This may explain the mechanistic divergence in ventilatory responses between adult NMRs and other adult mammals. In addition, several studies have demonstrated delayed/protracted developmental periods at the cellular level in NMRs (Penz et al., 2015; Peterson et al., 2012a). Together, these findings suggest that NMRs may be an ideal model in which to test the neotenic theory of hypoxia tolerance in an integrated fashion.

## Can we translate evolutionary hypoxia tolerance into clinical ischaemia tolerance?

From a biomedical perspective, the ultimate aim in studying naturally evolved mechanisms of hypoxia tolerance is the potential to translate these adaptations into hypoxia-intolerant species to protect against hypoxia-related pathologies, such as heart attack or ischaemic stroke in brain, and diseases and pathologies involving intermittent or chronic systemic hypoxaemia, such as sleep apnoea, anaemia or chronic pulmonary disorders (Little et al., 2021). Indeed, results from a handful of *in vitro* experiments in different hypoxia-tolerant species suggest that these organisms are highly resistant not only to environmental hypoxia but also to pathologies that result in reduced tissue oxygen, such as heart attack and ischaemic stroke (Christian et al., 2008; Dave et al., 2006; Nathaniel et al., 2009; Pamenter et al., 2012; Ross et al., 2006). Thus, hypoxia-tolerant mammals may offer a novel and impactful contribution to biomedical research. However, the translational potential for

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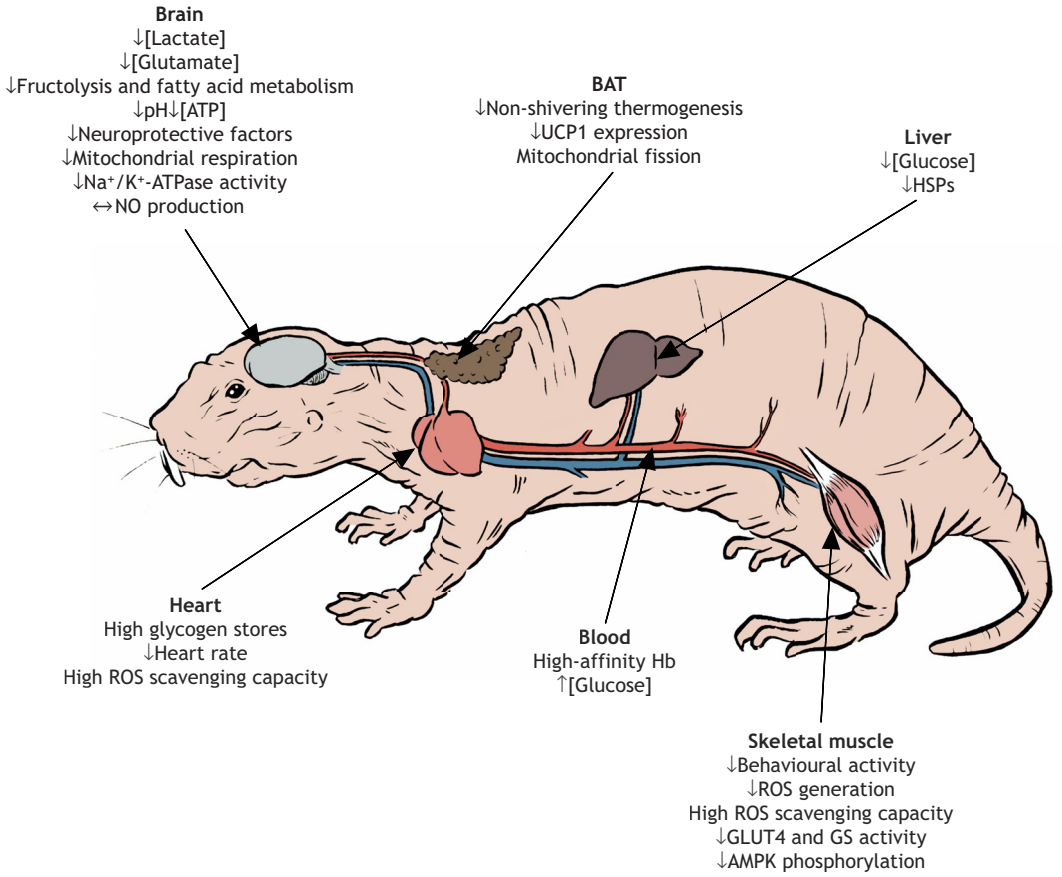


**Fig. 4. Systemic responses to acute hypoxia in NMRs.** Numerous cellular and molecular adaptations to acute and chronic hypoxia have been identified in NMRs. AMPK, AMP kinase; BAT, brown adipose tissue; GLUT4, glutamate transporter 4; GS, glycogen synthase; Hb, haemoglobin; HSPs, heat shock proteins; NO, nitric oxide; ROS, reactive oxygen species; UCP1, uncoupling protein 1. See text for references.

these models to inform therapeutic treatments has not yet been realized.

Beyond systemic hypoxia tolerance, there is considerable evidence that the NMR brain is tolerant of hypoxic and ischaemic stresses *ex vivo*. For example, NMR brain slices retain synaptic activity in anoxia for up to 30 min, tolerate oxygen–glucose deprivation (OGD) for 24 h, and exhibit blunted neuronal $Ca^{2+}$ influx during hypoxia (Larson and Park, 2009; Nathaniel et al., 2009; Peterson et al., 2012b). Conversely, hypoxia-intolerant murine brain slices tolerate only a few minutes of anoxia or OGD and exhibit large-scale deleterious $Ca^{2+}$ influx during hypoxia (Nathaniel et al., 2009; Peterson et al., 2012b). Furthermore, at the organelle level and relative to mice, NMR brain mitochondria better retain respiration capacity, have lower $H_2O_2$ emission rates and stable respiratory coupling ratios, and better maintain membrane integrity following *in vitro* ischaemia or ischaemia–reperfusion treatments (Cheng and Pamenter, 2021). These findings hint at a high potential for translational lessons from this species.

## Conclusions and future direction

Extreme environments are powerful drivers of novel adaptations, and this relationship is becoming particularly apparent in the study of NMRs. Living in a largely sealed subterranean environment and in a nearly unique eusocial colony structure, NMRs putatively experience irregular but potentially severe episodes of hypoxic stress. This has led to the development of a suite of adaptations that mitigate the effects of acute systemic hypoxaemia (Fig. 4). Many of these adaptations are shared with other hypoxia-tolerant mammals (e.g. decreased thermoregulation, mitochondrial remodelling), or with evolutionarily distinct vertebrate champions of anoxia tolerance (e.g. metabolic rate depression, synaptic arrest in brain, mitochondrial remodelling); however, several of these adaptations may be unique to NMRs (e.g. neotenic expression of proteins, cell types and synaptic signalling architecture in the central nervous system), supporting the hypothesis that this species has followed its own path in adapting to a challenging hypoxic niche.

Intriguingly, many of these adaptive traits are shared with neonates of other mammalian species, suggesting that the neotenic theory of hypoxia tolerance may underlie the evolution of these traits in NMRs. However, the general lack of information regarding cellular and physiological responses to hypoxia in neonatal and juvenile NMRs limits our ability to draw this conclusion intraspecifically, and considerable research is warranted into the ontogeny of this species to understand how NMRs respond to hypoxia at different developmental stages. In addition, more attention is needed towards in-depth study of adaptive mechanisms in a wider variety of hypoxia-tolerant mammals. Finally, it has long been speculated that the hypoxia tolerance of NMRs (and other tolerant vertebrates) may imbue them with tolerance to ischaemic stresses and the time is ripe for this question to finally be addressed in a rigorous manner.

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