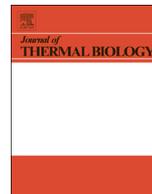




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Evaporative cooling and vasodilation mediate thermoregulation in naked mole-rats during normoxia but not hypoxia



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ABSTRACT

Naked mole-rats are among the most hypoxia-tolerant mammals but have a poor thermoregulatory capacity due to their lack of insulating fur and fat, and small body size. In acute hypoxia, naked mole-rat body temperature (T_b) decreases to ambient temperature (T_a) but the mechanisms that underlie this thermoregulatory response are unknown. We hypothesized 1) that naked mole-rat blood vessels vasodilate during hypoxia to shunt heat toward the body surface and/or 2) that they augment heat loss through evaporative cooling. Using open-flow respirometry (indirect calorimetry) we explored metabolic and thermoregulatory strategies of naked mole-rats exposed to hypoxia (7% O_2 for 1 h) at two relative humidities (RH; 50 or 100% water saturation), and in two T_a 's (25 and 30 °C), alone, and following treatment with the vasoconstrictor angiotensin II (ANGII). We found that T_b and metabolic rate decreased in hypoxia across all treatment groups but that neither RH nor ANGII effected either variable in hypoxia. Conversely, both T_b and metabolic rate were reduced in 100% RH or by ANGII treatment in normoxia at 25 °C, and therefore the absolute change in both variables with the onset of hypoxia was reduced when vasodilation or evaporative cooling were prevented. We conclude that naked mole-rats employ evaporative cooling and vasodilation to thermoregulate in normoxia and in 25 °C but that neither mechanism is involved in thermoregulatory changes during acute hypoxia. These findings suggest that NMRs may employ passive strategies such as reducing thermogenesis to reduce T_b in hypoxia, which would support metabolic rate suppression.

1. Introduction

Animals that inhabit hypoxic environments have evolved complicated suites of physiological adaptations that enable them to thrive in such low oxygen niches (Bickler and Buck, 2007; Buck and Pamerter, 2018; Dzal et al., 2015; Hochachka et al., 1996). The key to tolerating prolonged hypoxia is to match metabolic demand to reduced energy supply (i.e., reduced oxygen supply; Buck and Pamerter, 2006), and hypoxia-tolerant animals typically exhibit robust decreases in metabolic rate when oxygen supplies are limited (Dzal et al., 2015; Guppy and Withers, 1999; Hochachka, 1986). Conversely, hypoxia-intolerant animals are generally unable to sufficiently reduce their metabolic rate during hypoxia to accommodate reduced oxygen supply.

Thermoregulation is an energetically-expensive process,

particularly in small mammals, and many species employ thermoregulatory strategies to reduce body temperature (T_b) and facilitate reduced metabolic demand in acute and prolonged hypoxia. These thermoregulatory strategies can be roughly divided into three categories: 1) behavioural (e.g., reductions in huddling behaviour, seeking cooler environments, passive heat loss through heat transfer via direct skin contact with moist soil, etc. (Okrouhlik et al., 2015)), 2) circulatory (e.g., vasodilation or the evolution of morphological features within the circulatory system that facilitate heat loss, such as arteriovenous anastomoses that provide increased blood flow to the skin), and 3) decreasing thermogenesis (e.g., turning off non-shivering and shivering thermogenesis, downregulating mitochondrial function) (Bicego et al., 2007; Ramirez et al., 2007; Staples, 2016; Steiner and Branco, 2002). In addition, many animals employ radiative heat loss

Abbreviations: Angiotensin II, ANGI; RFID, radio frequency identification; RH, relative humidity; T_a , ambient temperature; T_b , body temperature; \dot{V}_{CO_2} , carbon dioxide production rate; \dot{V}_{O_2} , oxygen consumption rate

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and/or evaporative cooling through the evaporation of water molecules from the skin or surface membranes (e.g., sweating, panting) to prevent overheating. Similar processes may also facilitate decreases in T_b in hypoxia. For example, some reptiles spread urine on their skin to facilitate rapid heat loss in hypoxia (Tattersall and Gerlach, 2005). It is important to note that, although the cessation of active thermogenesis is the only process of the three categories described above that would confer direct energy savings, reductions of T_b through behavioural or circulatory means would nonetheless confer significant energy savings by systemically reducing the rate of cellular, molecular, and enzymatic activities through temperature-coefficient (Q_{10}) related energy savings.

Naked mole-rats (*Heterocephalus glaber*) are among the most hypoxia-tolerant mammals identified and tolerate minutes of complete anoxia, hours at 3% O_2 , and days to weeks at 8% O_2 (Chung et al., 2016; Pamerter et al., 2015, 2018; Park et al., 2017). The rate of oxygen consumption (\dot{V}_{O_2} ; an indirect measure of metabolic rate) of adult naked mole-rats decreases by up to 85% in severe hypoxia (3% O_2). Metabolism decreases by ~70% in 7% O_2 , which is the level of hypoxia employed in the present study (Pamerter et al., 2015, 2018, 2019). Although this degree of \dot{V}_{O_2} suppression is not remarkable among hypoxia-tolerant species (Guppy and Withers, 1999), it is important to note that other mammals that are capable of similar or more extreme metabolic rate suppression in severe hypoxia typically enter into a coma- or torpor-like state until oxygen levels are restored (Guppy and Withers, 1999; Hayden and Lindberg, 1970). Conversely, naked mole-rats remain awake and active in hypoxia, albeit to a reduced degree (Houlahan et al., 2018; Ilacqua et al., 2017; Kirby et al., 2018). Therefore, understanding physiological mechanisms that support reduced metabolic demand during hypoxic periods, despite the avoidance of torpor, is of interest in elucidating the underlying adaptations that support hypoxia-tolerance in this remarkable species.

Naked mole-rats are poor thermoregulators due to their lack of insulating fur and fat (Daly and Buffenstein, 1998), and their small body size (Sumner, 2019). In normoxia, and as a result of this poor ability to retain heat, naked mole-rats exhibit a mesothermic thermoregulatory phenotype in isolation such that at temperatures well below their thermoneutral zone, they are unable to effectively maintain thermal homeostasis. However, their metabolic rate increases substantially in the cold, which indicates that they do attempt to thermoregulate, even at substantial metabolic cost (Kirby et al., 2018; McNab, 1966; Withers and Jarvis, 1980). Naked mole-rats are able to ameliorate this cost to some degree in normoxia by moving to warmer environments (Kirby et al., 2018), by huddling to help conserve heat in their crowded natural burrow systems (Yahav and Buffenstein, 1991), or if they are provided with insulation (Withers and Jarvis, 1980). Both huddling and the provision of insulation decrease the amount of surface area exposed per animal and lower individual metabolic demand (\dot{V}_{O_2}) (Withers and Jarvis, 1980; Yahav and Buffenstein, 1991). These observations suggest that other physiological adaptations that reduce heat loss may also confer metabolic savings in hypoxia.

Recently, we have begun to explore thermoregulatory responses to acute hypoxia in naked mole-rats. During hypoxia, naked mole-rat T_b decreases to near ambient temperatures (T_a) (Ilacqua et al., 2017; Kirby et al., 2018; Pamerter et al., 2019), suggesting the realization of thermoregulatory-related energy savings. Our investigations to date have focused upon behavioural strategies and we have found that naked mole-rats do not employ behavioural thermoregulation *per se*. Specifically, naked mole-rats decrease overall behavioural activity in hypoxia but when given the option of choosing between different environmental temperatures when oxygen is limited, they prefer warm temperatures and avoid colder environments (Ilacqua et al., 2017; Kirby et al., 2018). Similarly, naked mole-rat huddling behaviour is unchanged in acute hypoxia (Houlahan et al., 2018). Taken together, these data suggest that naked mole-rats do not employ anapyrexia strategies in response to low environmental oxygen.

In the present study we sought to examine the second category of

potential thermoregulatory responses in hypoxia (i.e., circulatory strategies). Specifically, we comprehensively evaluated potential roles for peripheral vasodilation and evaporative cooling in thermoregulatory and metabolic responses to acute hypoxia. Arterioles and venules are connected via arteriovenous anastomoses in naked mole-rat dorsal skin, and thus capillary networks are brought close to the surface of the skin and are believed to mediate cooling of the blood in normoxia (Daly and Buffenstein, 1998). When the skin is instead chilled, the capillaries constrict, reducing the flow of blood to the surface of the skin and thereby conserving heat. These observations suggest that naked mole-rats could shunt blood to their skin while in hypoxia to dump heat and facilitate whole body metabolic cooling and thus reduce metabolic demand through the Arrhenius effect (Schulte, 2015). Conversely, naked mole-rats lack subcutaneous sweat glands and are therefore unable to utilize the common evaporative cooling strategy of sweating (Daly and Buffenstein, 1998). However, naked mole-rats may utilize moisture found in their environment or even bodily fluids to disperse heat through evaporative means in hypoxia (Tattersall and Gerlach, 2005).

We hypothesized that naked mole-rats utilize circulatory strategies to rapidly decrease T_b in acute hypoxia and predicted that abrogation of these abilities by injection of a vasoconstrictor (angiotensin II, ANGII) and/or exposure to an H_2O -saturated environment (100% relative humidity, RH), respectively, would impair their ability to reduce T_b , and in turn metabolic rate, in acute hypoxia. To test our hypothesis, we exposed naked mole-rats to 1 h of hypoxia (7% O_2) at two ambient temperatures (T_a 's; 25 and 30 °C), in either 50 or 100% RH, and also following injection of ANGII, and measured metabolic rate (O_2 consumption and CO_2 production, \dot{V}_{O_2} and \dot{V}_{CO_2} , respectively) and T_b .

1.1. Materials and methodology

Animals. Naked mole-rats were group-housed in interconnected multi-cage systems at 30 °C and 21% O_2 in 50% humidity with a 12L:12D light cycle. Animals were fed fresh tubers, vegetables, fruit, and Pronutro cereal supplement *ad libitum*. Animals were not fasted prior to experimental trials. All experimental procedures were approved by the University of Ottawa Animal Care Committee in accordance with the Animals for Research Act and by the Canadian Council on Animal Care. All experiments were performed during daylight working hours in the middle of the animals' 12L:12D light cycle. Naked mole-rats that are housed within colony systems do not exhibit circadian rhythmicity of general locomotor activity (Riccio and Goldman, 2000b), and exhibit inconsistent rhythmicity of T_b and metabolic rate (Riccio and Goldman, 2000a); however, significant changes in these latter parameters were only reported in animals during the nocturnal phase of their circadian cycle with no significant changes observed during the daylight period of this cycle. Therefore, since we only ran experimental trials during the daylight period, we do not expect our results to be influenced by circadian rhythms. We examined physiological responses to environmental hypoxia in non-breeding naked mole-rats that were 1–2 years old. Non-breeding (subordinate) naked mole-rats do not undergo sexual development or express sexual hormones and thus we did not take sex into consideration when evaluating our results (Holmes et al., 2009).

Experimental Design. Seventy (70) male and female subordinate adult naked mole-rats weighing 47.2 ± 6.9 g (mean \pm s.d.) were divided into the following 9 experimental groups: (i) 30 °C + 100% RH ($n = 8$), (ii) 30 °C + 100% RH + ANGII ($n = 8$), (iii) 30 °C + 0% RH ($n = 8$), (iv) 30 °C + 0% RH + sham injection ($n = 6$), (v) 30 °C + 0% RH + ANGII ($n = 8$), (vi) 25 °C + 0% RH ($n = 8$), (vii) 25 °C + 0% RH + ANGII ($n = 8$), (viii) 25 °C + 100% RH ($n = 8$), and (ix) 25 °C + 100% RH + ANGII ($n = 8$). For sham injection and ANGII treatment groups, animals received one intraperitoneal injection of either saline or ANGII ($25 \mu\text{g ml}^{-1}$, total volume $\sim 250 \mu\text{L}$; Sigma Aldrich, USA). Intraperitoneal delivery of ANGII has been shown to increase vasomotor sympathetic drive for at least 2 h post-injection in

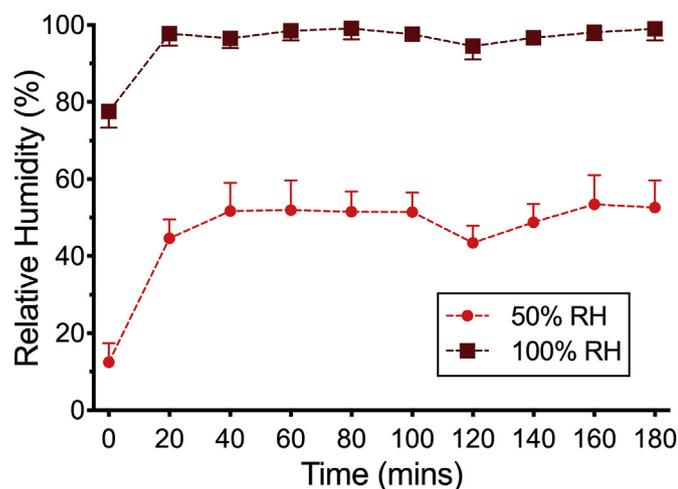


Fig. 1. Chamber relative humidity. Summary of chamber relative humidity at 30 °C from experiments in which animals were supplied dry (light red circles) or water-bubbled gasses (dark red squares). Data are mean \pm s.d. from 12 experiments each.

other rodents (Zubcevic et al., 2017). Injections did not appear to impact the animals negatively in that they remained alert and active following injection and did not exhibit any signs of pain or discomfort.

At the start of the experiment (and following injection if appropriate), naked mole-rats were placed into a 500 ml cylindrical experimental chamber. All animals urinated and defecated shortly after being placed into the experimental chamber and the addition of moisture from this waste, combined with the low flow rate of gas through the chamber (see below), increased the RH from 0% (incurrent gas) to ~50% (actual excurrent gas). Therefore, we considered our 0% RH data as being 50% saturated for the purpose of our data presentation and discussion (Fig. 1). Baseline recordings were obtained for 1 h in normoxia and then the incurrent gas composition was switched to 7% O₂ for 1 h followed by 1 h in normoxia (recovery). Following experimentation, animals were returned to their colonies. Experiments were conducted in environmental rooms held at 25 or 30 °C and animals were acclimated for 2–3 h at the appropriate temperature prior to commencing experimentation. These temperatures were selected since an T_a of 30 °C is the housing temperature of our colonies, and is near the thermoneutral zone of naked mole-rats (which spans from ~30.5 to 34 °C (Yahav and Buffenstein, 1991)); the 25 °C temperature was selected to increase the thermal scope within which the animals were able to respond through thermoregulatory adaptations to hypoxia. Naked mole-rats have a higher metabolic rate in colder temperatures relative to near their thermoneutral zone (Ilacqua et al., 2017; Kirby et al., 2018; McNab, 1966; Withers and Jarvis, 1980), and thus repeating our experiments in this temperature magnified the impact of our treatments on metabolic rate and T_b, and therefore our ability to detect any physiological changes in this condition.

Flow-through respirometry. The animal chamber was sealed and constantly ventilated with gas mixtures set to the desired fractional gas composition by calibrated rotameters (Praxair, Mississauga, ON, CA). The advantage of this open-flow system is that it prevents the depletion of O₂ and accumulation of metabolic CO₂ by flushing the animal chamber with fresh gas, and it allows for continuous and simultaneous monitoring of metabolic and ventilatory variables. Inflowing gas was provided at a flow rate of 85 ml min⁻¹, as assessed by a calibrated mass flow meter (Q-G266 Flow Monitor, Qubit Systems). The gas flowing into the chamber first passed through either a bubbler or a drying column containing Drierite desiccant to achieve the conditions of ~100% or 0% RH, respectively. The bubbler or drying column was joined to the experimental chamber via the outflow tube. After passing through the chamber, the outflowing gas traveled to the inflow tube of

a relative humidity sensor (RH-200 RH/Dewpoint Meter, Sable Systems Int., Las Vegas, NV, USA) and then through a drying column before entering a series of gas analyzers. The gas first passed through the flow analyzer, followed by the O₂ analyzer (Q-S102, Qubit Systems) and finally, the CO₂ analyzer (Q-S153, Qubit Systems). Gas analyzers were calibrated prior to each trial with 20.95% O₂, 1.5% CO₂, balanced with N₂, and with 100% N₂ gas mixes. The \dot{V}_{O_2} and \dot{V}_{CO_2} were calculated using equations 11.7 and 11.8 from (Lighton, 2008), and accounting for time lag of gas flow between the O₂ and CO₂ sensors. All metabolic variables are reported at standard temperature, pressure, dry (STPD).

Body temperature. Body temperature was measured using a handheld radio frequency identification (RFID) reader that scanned individual naked mole-rats instrumented with subcutaneous RFID microchips (Destron Fearing, Dallas, TX). The first measurement was taken immediately after placing the animal into the chamber and then subsequent measurements were taken every 10 min, as described previously (Ilacqua et al., 2017; Kirby et al., 2018). Measurements were taken when the body region containing the RFID microchip was not in contact with the chamber surface to avoid biased readings. The accuracy of these microchips for measuring T_b was confirmed in a separate set of experiments in which we took core T_b measurements using a thermocouple (Thermalert Model TH-8 temperature monitor, Physitemp, Clifton, NJ, USA) from animals held at 30 °C (n = 7). Temperatures measured by RFID vs. rectal probe were not significantly different (T_{b(microchip)} = 32.34 °C, T_{b(rectal)} = 32.42 °C).

Data collection and statistical analysis. Ambient temperature and incurrent and excurrent O₂ and CO₂ concentrations were recorded and analysed using Loggerpro software (Vernier, USA). We determined average T_a, T_b, \dot{V}_{O_2} , \dot{V}_{CO_2} , and RH values for the last 10–15 min of each O₂ exposure (21% and 7% O₂). Inflowing gas concentrations were measured before and after each O₂ exposure. Gas flow was measured continuously throughout all experiments. Statistical analysis was performed to determine the effects of O₂ level, T_a, RH, and ANGII injection. Statistical significance was determined using a two-way (treatment and O₂ level) repeated measures analysis of variance (RM ANOVA) to analyze the final 10 min of each experimental stage (normoxia and hypoxia) (see statistical table in supplementary materials section). For comparisons between the magnitude of change between normoxia and hypoxia at T_a's of 25 and 30 °C, significance was evaluated using an ordinary one-way ANOVA. Dunnett's multiple comparison test was performed within groups while Tukey's multiple comparison test was performed between groups. All physiological and behavioural variables met the assumptions of normality, homogeneity of variances, linearity, and independence, and residuals from the statistical models were confirmed for normality. All results are presented as mean \pm s.d., with statistical significance set as $\alpha < 0.05$.

2. Results

Body temperature and metabolic rate are significantly reduced in acute hypoxia near the thermoneutral zone. Body temperature, \dot{V}_{O_2} , and \dot{V}_{CO_2} were first measured at 30 °C, which is the temperature at which our animal colonies are housed, to assess the effects of acute hypoxia on thermoregulation and metabolic rate near the naked mole-rat thermoneutral zone. Changes in T_b, recorded every 10 min throughout the experimental period are presented in Fig. 2A (n = 6 for 50% RH + saline (sham injections) and n = 8 for all other treatments). Analysis with a 2-way repeated measures ANOVA revealed a significant effect of acute hypoxia on T_b for all experimental treatments (Fig. 2B, see table in supplemental materials for all 2-way RM ANOVA values). Conversely, there was no significant difference between groups within either normoxic or hypoxic conditions (Fig. 2B), although there was a significant interaction between treatment and oxygen exposure. A subsequent one-way repeated measures ANOVA indicated that there were no effects on the magnitude of change in T_b in acute hypoxia (Fig. 2C). Specifically, T_b was ~32 °C in normoxia and decreased by

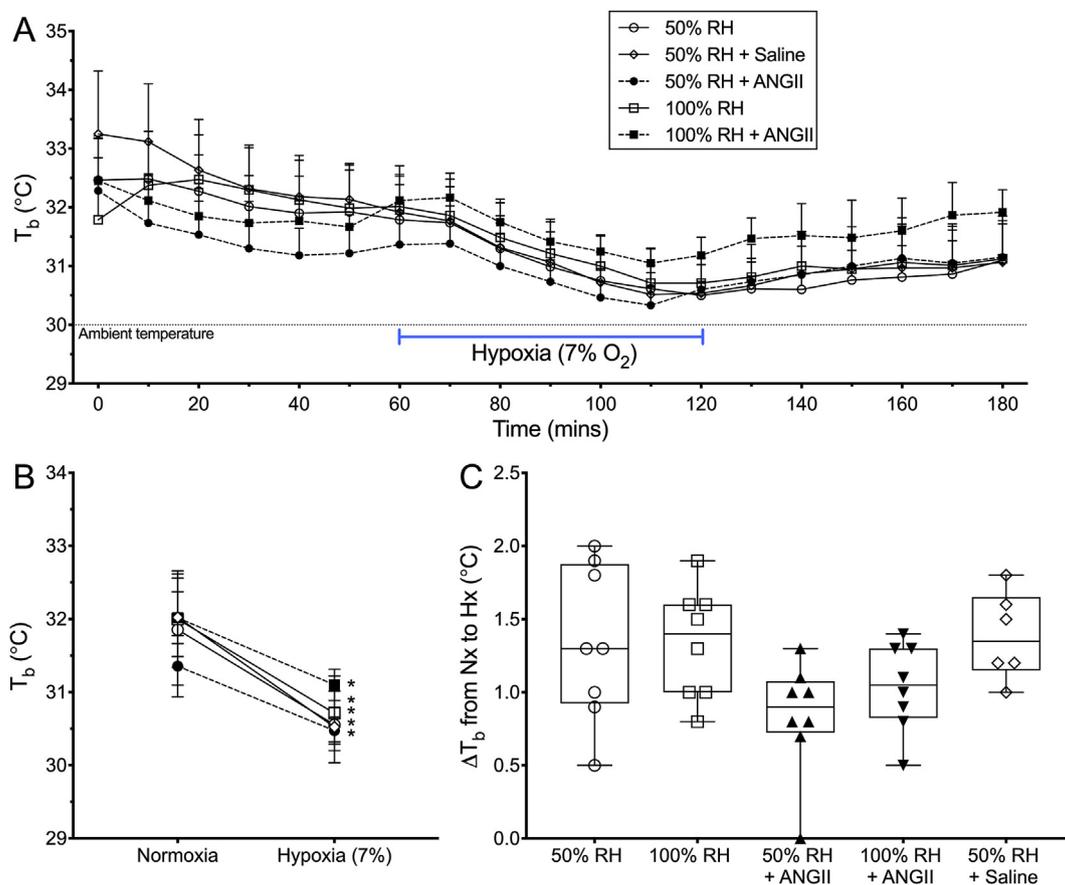


Fig. 2. Naked mole-rats exhibit decreases in body temperature in acute hypoxia near their thermoneutral zone. Untreated naked mole-rats or naked mole-rats injected with either saline (sham) or the vasoconstrictor angiotensin II (ANGII) were placed in a metabolic chamber held at 30 °C and exposed to 60 min periods of normoxia (21% O₂, control) and hypoxia (7% O₂) and normoxic recovery (21% O₂) in either 50 or 100% relative humidity (RH). **(A)** Body temperature (T_b) of individuals were recorded every 10 min throughout the experiment. Data are mean \pm s.d. for $n = 8$ individuals for all treatment groups except sham injections, for which $n = 6$. Dotted line indicates ambient temperature. **(B)** Summary of the last 10 min of normoxia and hypoxia exposures from panel A presented as mean \pm s.d. **(C)** Summary of ΔT_b from normoxia to hypoxia presented as box (95% confidence interval) and whiskers (range of data) with mean and individual data points. Asterisks (*) indicate significant differences from normoxia to hypoxia ($p < 0.05$, 2-way repeated measures ANOVA with Tukey's multiple comparison test).

~ 1.5 °C during acute hypoxia in the saline, 50% and 100% RH control groups, and by ~ 1.0 °C in the ANGII-treated animals (Fig. 2C).

Similarly, both \dot{V}_{O_2} , and \dot{V}_{CO_2} were significantly reduced in acute hypoxia relative to normoxic controls in all treatment groups (Fig. 3A for \dot{V}_{O_2} and Fig. 3B for \dot{V}_{CO_2}). However, there was no significant difference between groups within either normoxia or hypoxia for either variable, and no significant interaction effects between treatment and oxygen level (see supplemental Table).

Vasodilation mediates the hypoxic change in body temperature and metabolic rate in a colder temperature. The naked mole-rat T_b is very close to the experimental temperature when held near the thermoneutral zone of this species and this provides minimal scope for thermoregulatory responses in acute hypoxia. Therefore, since analysis of our findings near the thermoneutral zone temperature suggested a potential effect of ANGII treatment on T_b without revealing specific differences between treatment groups, we repeated our experiments at a colder temperature (25 °C) to better resolve the effects of RH and vasodilation on thermoregulation in normoxia and hypoxia (Fig. 4A; $n = 8$ for all treatments).

Similar to in the warmer temperature, analysis with a 2-way repeated measures ANOVA revealed a significant effect of acute hypoxia on T_b for all experimental treatments (Fig. 4B). Furthermore, our analysis also revealed a significant interaction within normoxia, but not hypoxia (see supplemental Table). Notably, a Tukey's *post-hoc* test revealed that the 50% RH group was significantly different from all other groups in normoxia ($p = 0.0450$ vs. 100% RH, and $p < 0.0001$ 50%

RH + ANGII and 100% RH + ANGII). In addition, a one-way repeated measures ANOVA revealed a significant treatment effect of ANGII on the change in T_b in acute hypoxia (Fig. 4C), and Tukey's multiple comparison *post-hoc* test detected a significant effect of ANGII treatment in both 50 and 100% RH groups ($p = 0.0075$ for both). Specifically, T_b decreased by ~ 3.0 °C during acute hypoxia in the 50% and 100% RH control groups, and this change was decreased by ~ 50 –70% in ANGII-treated animals, primarily due to an ANGII-mediated decrease in normoxic T_b , which diminished the scope for change in hypoxia (Fig. 4C).

The changes in both \dot{V}_{O_2} , and \dot{V}_{CO_2} in 25 °C mirrored those of T_b in this colder temperature and both variables were significantly reduced in acute hypoxia relative to normoxic controls in all treatment groups (Fig. 5A for \dot{V}_{O_2} and Fig. 5B for \dot{V}_{CO_2}). There were also significant differences between treatment groups within normoxia but not hypoxia. Similar to our T_b results, a Tukey's multiple comparison *post-hoc* test indicated that the 50% RH group was significantly different from all other groups in normoxia ($p = 0.0330$ vs. 100% RH, and $p < 0.0001$ 50% RH + ANGII and vs. 100% RH + ANGII). In addition, ANGII treatment significantly decreased \dot{V}_{O_2} in the 100% RH group ($p = 0.0476$). For \dot{V}_{CO_2} , our results were less robust and here the effect of ANGII treatment on normoxic \dot{V}_{CO_2} was only significant for the 50% RH group ($p < 0.0001$). The effect of humidity (100% vs. 50% RH) was not significant ($p = 0.0877$).

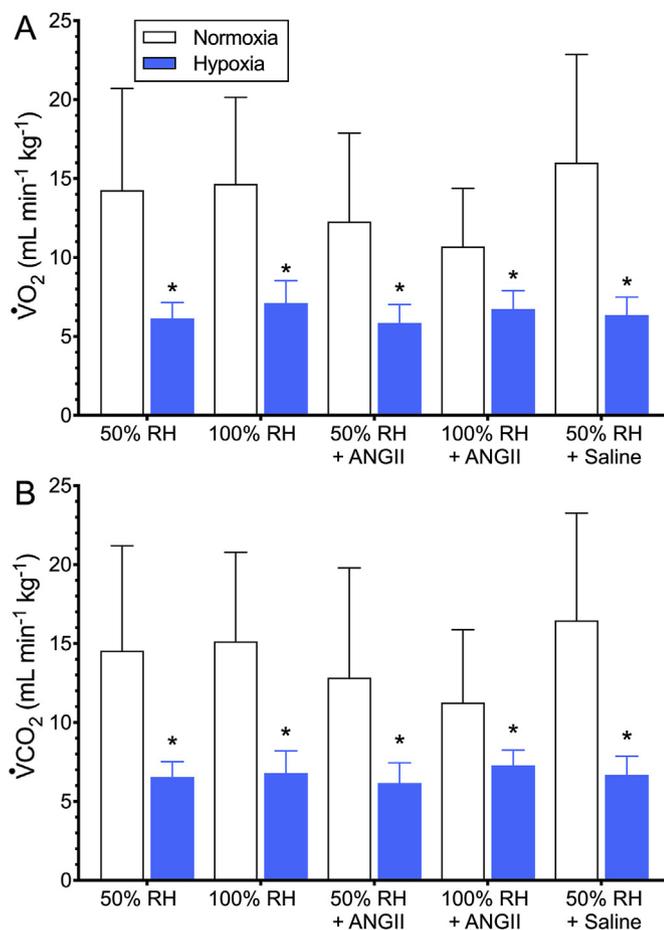


Fig. 3. Naked mole-rats exhibit decreases in metabolic rate during acute hypoxia near their thermoneutral zone. Summaries of (A) oxygen consumption rates (\dot{V}_{O_2}) and (B) carbon dioxide production rates (\dot{V}_{CO_2}) from naked mole-rats treated as in Fig. 1. Data are mean \pm s.d. Asterisks (*) indicate significant differences from normoxia to hypoxia ($p < 0.05$, 2-way repeated measures ANOVA with Tukey's multiple comparison test).

3. Discussion

Naked mole-rats exhibit a poor thermoregulatory capacity due to their lack of insulating fur and fat (Daly and Buffenstein, 1998), and due to their small body mass (Sumner, 2019). We hypothesized that this functional deficit would in fact be beneficial in hypoxia and that evaporative cooling and vasodilation would facilitate heat loss and support metabolic rate suppression. We manipulated RH, T_a , and vascular tone to explore the roles of evaporative cooling and vasodilation on thermoregulatory and metabolic strategies used by naked mole-rats in both normoxia (21% O_2) and hypoxia (7% O_2). We report several important findings. First, at a T_a near the naked mole-rat thermoneutral zone, blockade of either evaporative cooling or circulatory strategies have no effect on T_b or metabolism in either normoxia or hypoxia. Conversely, in a colder temperature in normoxia, the T_b and metabolic rate of naked mole-rats are dependent on the availability of both evaporative cooling and circulatory strategies such that abrogation of either of these thermoregulatory mechanisms impacts T_b and metabolic rate. Conversely, during acute hypoxia, T_b decreases to near T_a and metabolic rate decreases substantially in both experimental temperatures. The decreases in these two variables are consistent with several previous studies in this species (Chung et al., 2016; Dzal et al., 2019; Houlihan et al., 2018; Ilacqua et al., 2017; Kirby et al., 2018; Pamerter et al., 2014, 2018, 2019, 2015). Importantly however, the absolute level to which T_b and metabolic rate decrease during hypoxic exposure

in the present study is not dependent on either evaporative cooling or vasodilation thermoregulation mechanisms since blockade of either system has no effect on the absolute levels of T_b and metabolic rate in acute hypoxia in either experimental temperature.

Effects of RH on naked mole-rat thermoregulatory strategies. Several recent studies from our laboratory demonstrate that naked mole-rats alter their thermoregulatory profile in response to acute hypoxia such that their T_b decreases to $\sim T_a$ when oxygen is limited (Houlihan et al., 2018; Ilacqua et al., 2017; Kirby et al., 2018; Pamerter et al., 2019). However, the mechanism(s) underlying this thermoregulatory response are unknown. The RH of naked mole-rat burrows ranges between ~ 31 and 93% (Holtze et al., 2018), and thus the natural habitat of this species provides some scope in which to utilize an evaporative cooling strategy. However, physiological characteristics of this species have previously been thought to limit their capacity to utilize evaporative cooling strategies such as sweating and panting (Buffenstein and Yahav, 1991; Daly and Buffenstein, 1998).

Our study is the first to test a potential role for this strategy during hypoxia in this species and we report a significant effect of RH on both T_b and metabolic rate in normoxia at 25 °C (but not at 30 °C). It is likely that naked mole-rats in the 50% RH group lose body heat due to evaporative cooling during normoxia and thus the higher metabolic rate in this condition reflects active thermogenesis in this relatively cool temperature. Paradoxically, this thermogenesis apparently results in these animals having a higher T_b than in the 100% RH group, within which the animals cannot lose heat to evaporative cooling and may therefore maintain a T_b that is closer to T_a due to reduced thermogenesis-linked metabolic demand. This paradox is likely due to the poor insulative capacity of this species, which may result in a temporal uncoupling between heat generation and thermal homeostasis such that T_b may overshoot or undershoot the T_b set point when thermoregulation is modified by external factors (e.g., RH). Thus, in the 50% RH group, thermogenesis to offset heat loss due to evaporative cooling drives a higher metabolic rate (and thus T_b) than in the 100% RH group in which heat loss due to evaporative cooling is abrogated and therefore thermogenesis is reduced (along with metabolic rate and T_b). Such an uncoupling between metabolic rate and T_b has also been reported in cold-treated armadillos (Boily and Knight, 2004), which have an atypical thermoregulatory and metabolic profile (Boily, 2002), as do naked mole-rats. Alternatively, skin vascularization in the 50% RH group may be higher than in the 100% RH group as a means of retaining moisture when water is readily available.

With the onset of hypoxia, T_b and metabolic rate decrease in tandem but these changes are not affected by ambient RH levels. This suggests that the mechanism underlying the decrease in T_b in hypoxia in this species is a decrease in thermogenesis as opposed to active heat dumping due to vasodilation and evaporative cooling. This is a sensible strategy in hypoxia as decreasing thermogenesis would reduce metabolic demand, whereas shunting blood to the skin would require active regulation of the circulatory system, which would in turn require some degree of energy expenditure. Indeed, lowering T_b during hypoxia is a common survival response in small mammals (Barros et al., 2001; Wood and Gonzales, 1996; Wood and Stabenau, 1998), because reducing T_b reduces oxygen demand, and thus, small mammals typically decrease T_b to as low a value as they can tolerate based on their thermal and energetic needs (Hill, 1959). In the case of naked mole-rats, who live in warm and humid burrow systems (Holtze et al., 2018), the scope for such a thermoregulatory response is quite limited relative to that of other small mammals; however, naked mole-rats still appear to utilize this response to the extent to which they are able (Houlihan et al., 2018; Ilacqua et al., 2017; Kirby et al., 2018).

Effects of ANGII on naked mole-rat thermoregulatory strategies. Most mammals actively dump heat with the onset of acute hypoxia in order to facilitate rapid cooling and metabolic savings when oxygen is limited. This process is a means to support the regulated lowering of the T_b set point in hypoxia (Tattersall and Milsom, 2009), and typically

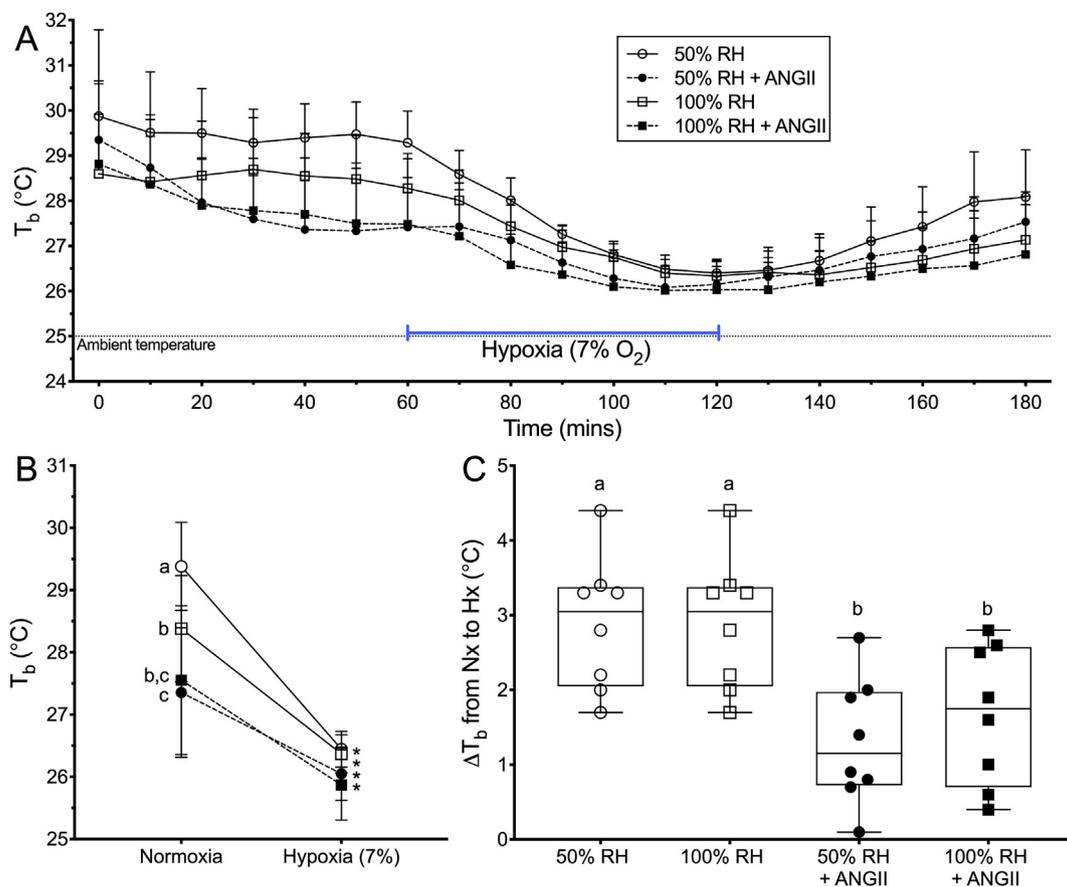


Fig. 4. Vasodilation mediates body temperature in normoxia and reduces the hypoxic change in body temperature in a cool temperature. Untreated naked mole-rats or naked mole-rats injected with the vasoconstrictor angiotensin II (ANGII) were placed in a metabolic chamber held at 25 °C and exposed to 60 min periods of normoxia (21% O₂, control) and hypoxia (7% O₂) and normoxic recovery (21% O₂) in either 50 or 100% relative humidity (RH). (A) Body temperature (T_b) of individuals were recorded every 10 min. Dotted line indicates ambient temperature. Data are mean ± s.d. for n = 8 individuals for all treatment groups. (B) Summary of the last 10 min of each exposure from panel A presented as mean ± s.d. (C) Summary of ΔT_b from normoxia to hypoxia presented as box (95% confidence interval) and whiskers (range of data) with mean and individual data points. Asterisks (*) indicate significant differences from normoxia to hypoxia, lower case letters denote differences between treatment groups (p < 0.05, 2-way repeated measures ANOVA with Tukey's multiple comparison test).

involves increased vasodilation of peripheral blood flow to shift heat away from the body core and enhance heat loss through evaporative and radiative means. This strategy has been observed in measurements of increased skin temperature, heat loss (through calorimetry), and/or increased peripheral circulation during acute hypoxia in a wide range of hypoxia-tolerant and hypoxia-intolerant mammals, including dogs (Britton, 1984), golden-mantled ground squirrels (Tattersall and Milsom, 2003), rabbits (Iriki and Kozawa, 1976), rats (Gordon, 1997), marmosets (Tattersall et al., 2002), and humans (Simmons et al., 2007), among others. In these species, such vasodilation represents an active thermoregulatory practice. Conversely, in naked mole-rats, injection of a vasoconstrictive agent (ANGII) does not impair the hypoxic decrease in T_b, suggesting that this change is a passive process, likely mediated by a switching off of thermogenesis.

Although passive heat loss as a mechanism of thermoregulation in mammals exposed to hypoxia has been suggested previously (Gordon, 1997; Mortola, 1993), experimental evidence overwhelmingly supports active heat loss in this paradigm. Therefore, our observations in naked mole-rats suggest that this species may be an outlier among mammals in their apparent use of slower, passive heat loss during acute hypoxia. Of course, naked mole-rats lack insulating fur and fat (Daly and Buffenstein, 1998), which is rare among mammals, and thus they are better suited to utilize a passive cooling strategy as they are able to lose heat passively much more rapidly than are most mammals.

Conclusions. Among mammals, naked mole-rats are remarkably hypoxia-tolerant but are poor thermoregulators. Within their natural

burrow systems, naked mole-rats have limited scope within which to respond to hypoxia through thermoregulatory means; however, they appear to utilize what little scope is available to them. The present study suggests that, unlike most mammals, naked mole-rats do not use active thermoregulation in acute hypoxia, but instead rely on passive heat dissipation to reduce T_b. This approach would have the added benefit of conserving energy relative to the metabolic cost of active thermoregulation in hypoxia. This strategy is likely supported by the poor thermal retention of this species, which is due to a lack of fur, minimal deposits of subdermal fat, and small body size. Such a passive drop in T_b nonetheless suggests that naked mole-rats reduce active thermogenesis in acute hypoxia and further studies are warranted to investigate this possibility.

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Conflicts of interest

We have no competing interests.

Author contributions

MP and GT conceived of and designed the study. AV and AZ

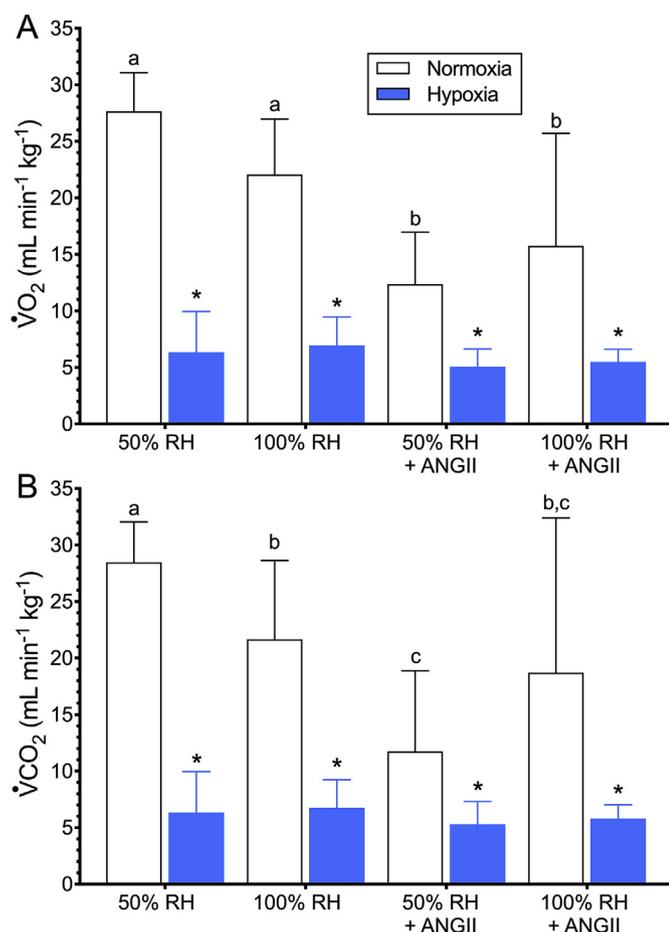


Fig. 5. Vasodilation mediates metabolic rate in normoxia and reduces the hypoxic metabolic response in a cool temperature. Summaries of (A) oxygen consumption rates ($\dot{V}O_2$) and (B) carbon dioxide production rates ($\dot{V}CO_2$) from naked mole-rats treated as in Fig. 3. Data are mean \pm s.d. Asterisks (*) indicate significant differences from normoxia to hypoxia, lower case letters denote differences between treatment groups ($p < 0.05$, 2-way repeated measures ANOVA with Tukey's multiple comparison test).

performed the physiology experiments. AV and MP analysed the data. MP conducted statistical analysis and MP, GT and AV wrote the manuscript. MP, GT, AV, and AZ edited the manuscript, gave final approval of the published version and agree to be accountable for all content therein. AK trained AV in the indirect calorimetry technique and provided logistical support to AV but did not make a direct contribution to this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2019.07.011>.

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