

Non-shivering thermogenesis in four species of African mole-rats differing in their sociality

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Abstract

Many mammalian species are adapted to living in burrows for most of their lives. Inhabiting such thermally stable environments may influence the variation on the species thermogenic capacity, particularly non-shivering thermogenesis (NST). African mole-rats (*Bathyergidae*) are subterranean rodents occurring in fynbos, grassland and wooded savannas across sub-Saharan Africa that vary in the complexity of their social systems, ranging from strictly solitary to highly social species. The presence and magnitude of NST are well known in social bathyergids, but no such data exist for their solitary counterparts. In this study, we quantified NST in three solitary mole-rat species represented by three distinct genera together with one social species. Our results showed that NST in all species is functional. Maximum metabolic rate after norepinephrine injection was equivalent to 269% of resting values in the social giant mole-rat *Fukomys mechowii* and 166%, 282% and 157% in the three solitary species: the silvery mole-rat *Heliophobius argenteocinereus*, the Cape mole-rat *Georychus capensis* and the Cape dune mole-rat *Bathyergus suillus*, respectively. To test our prediction that NST capacity is higher in solitary bathyergids, we combined our data with those available for other members of this family. In contrast to our prediction, NST did not differ between social and solitary bathyergids. Body mass, as the main factor, and minimum air temperature (T_{\min}), accounts for more than 80% of NST variation in bathyergid mole-rats.

Introduction

Endotherms increase resting metabolic thermogenesis to balance heat loss and thereby maintain a stable body temperature (T_b) when ambient temperature (T_a) decreases below the lower critical limit of thermoneutrality (Withers *et al.*, 2016). Maintenance of a stable T_b under these conditions involves physiological mechanisms that increase heat production above the minimum, obligatory and continuous heat production (i.e., obligatory thermogenesis). Basal metabolic rate can be used as an indicator of obligatory thermogenesis, whereas shivering (ST) and non-shivering thermogenesis (NST) are components of the total thermogenic capacity of an individual that provides heat at low T_a s (Hohtola, 2004; Cannon & Nedergaard, 2011). Shivering uses muscle contractions to produce heat, while mammalian NST involves heat generation through molecular decoupling in brown adipose tissue (BAT) cells (Cannon & Nedergaard, 2004; Hohtola, 2004). In particular, it has been suggested that ST requires more energy than NST because it involves muscular activity (Janský, 1973). Also, NST is

considered the most plastic element of the heat production machinery (Cannon & Nedergaard, 2004). Adaptive changes in thermogenic variables, particularly NST, are critical for survival in environments that exhibit pronounced seasonal variations of T_a . For example, in surface-dwelling mammals, NST usually increases more than 150% after winter acclimatization (Wunder & Gettinger, 1996; Roberts, 2011).

About 300 mammalian species are well adapted to a life in underground burrows, the occupancy of which reduces both daily and seasonal fluctuations of T_a compared to aboveground microsites (Bennett, Jarvis & Davies, 1988; Burda, Šumbera & Begall, 2007). Because seasonal fluctuations in T_a are buffered in underground burrows compared to on the surface, thermogenic capacity may be expected to be less pronounced in fossorial taxa. In surface species, the energy cost of NST increases several times after prolonged cold exposure (Nespolo *et al.*, 1999; Van Sant & Hammond, 2008); thus, from an evolutionary perspective, buffered thermal conditions in the below-ground environment should be reflected in reduced capacity for NST. Indeed, in several species of the South American

fossorial *Ctenomys*, NST is lower than expected when compared to surface-dwelling congeners of comparable body mass (Luna *et al.*, 2012, 2019). In this genus, the contribution of NST to total thermogenic capacity varied between species, from a combination of both NST and ST (e.g., *C. porteusi*) to almost an exclusive contribution of ST (e.g., *C. australis*; Luna *et al.*, 2019). In a social fossorial species from South America, the coruro (*Spalacopus cyanus*), NST is the sole heat-producing mechanism (Nespolo *et al.*, 2001). Nevertheless, all these species leave their burrows to feed aboveground close to their burrow openings (see Begall *et al.*, 2007), complicating the differentiation of the influence of thermal conditions on the surface from those prevailing in burrows on thermogenic capacity, in particular NST (Luna *et al.*, 2019).

Fossorial rodents vary in their degree of adaptations to the use of burrows. Species are usually denoted as strictly fossorial or subterranean when they live almost permanently belowground (Begall *et al.*, 2007). In evolutionary terms, aboveground thermal conditions can thus have a negligible effect on their NST, allowing us to partition the effects of aboveground and burrow environment (T_a s) when comparing species with different lifestyles. African mole-rats (Bathyergidae) are subterranean rodents inhabiting various habitats in sub-Saharan Africa from sea level up to high mountains. They feed primarily on belowground storage organs of plants, and rarely, if ever, leave their burrow systems. Interestingly, bathyergids exhibit certain social complexity, ranging from strictly solitary through to highly social species (Bennett & Faulkes, 2000). Non-shivering thermogenesis has already been studied in several social bathyergid species, with these studies revealing that NST is well-developed and its magnitude correlated with a species' ecological traits. Activation of NST by an injection of norepinephrine (NE) increases the metabolic rate by about 4.5 times and T_b by 5.4°C in the Highveld mole-rat *Cryptomys hottentotus pretoriae* (Haim & Fairall, 1986). Similarly, in the Damaraland mole-rat *Fukomys damarensis*, the metabolic rate increased two-fold and T_b showed a 4°C increase (Hislop & Buffenstein, 1994). Both species live in relatively cold areas of southern Africa. In the same way, the metabolic rate of the Lesotho mole-rat *Cryptomys hottentotus mahali*, from grasslands at an elevation of 1600 m.a.s.l., increased around 2.8 times after the injection of NE. Individuals of the same species that inhabit grasslands at 3200 m.a.s.l. showed a higher metabolic rate increase (~3.6 times) than low-elevation counterparts (Broekman *et al.*, 2006). The increase in metabolism was also high (3.5 times) in the naked mole-rat *Heterocephalus glaber*, a species from the arid Afrotropics. The increase in T_b was less than 3°C, probably due to rapid heat loss associated with its small body mass and virtually hairless body surface (Woodley & Buffenstein, 2002). In this species, the presence of anatomically similar BAT to that found in homeothermic mammals (Daly, Williams & Buffenstein, 1997), and hence functional NST, is surprising because this species is thought to occupy burrows with stable and high T_a , near their thermoneutral zone [TNZ: $T_a \sim 32^\circ\text{C}$; Bennett *et al.*, (1988), but see Holtze *et al.*, (2018) for large fluctuations in surface T_b and lower values of T_a].

Until now, African mole-rat NST has been investigated only in social species, which may reduce individual heat loss by huddling with conspecifics, thereby obviating the need for pronounced NST. No comparable studies have focused on the potential for NST in solitary mole-rats (i.e., species that do not take advantage of social thermoregulation). In our present study, we focused on NST capacity in several species of African mole-rats differing in their degree of sociality. We measured NST capacity in three solitary species representing three genera, the silvery mole-rat *Heliophobius argenteocinereus*, the Cape dune mole-rat *Bathyergus suillus*, and the Cape mole-rat *Georchus capensis* and one social species, the giant mole-rat *Fukomys mechowii*. By combining our new data with published data for representative species of social *Cryptomys*, *Fukomys* and *Heterocephalus*, we were able to evaluate NST capacity across the family Bathyergidae. We hypothesized that NST is lower in social species than in solitary ones because of the potential use of social thermoregulation. In addition, we also tested the effect of body mass and several climatic characteristics that may play a role in influencing the presence and magnitude of NST.

Materials and methods

Animal husbandry

The silvery mole-rat is a solitary species, weighing 120–330 g, which lives in grassland and woodland from Southern Kenya to Northern Mozambique (Šumbera, Chitaukali & Burda, 2007a). The solitary Cape dune mole-rat is the largest mole-rat species weighting ~950 g (males) and ~780 g (females) inhabiting sandy soils along the southwestern coast of South Africa (Bennett *et al.*, 2009). The solitary Cape mole-rat (~180 g) occupies mesic areas of the South Africa, with most of the distribution in southwestern part of the country, but some populations occurs in montane fynbos (Bennett, Maree & Faulkes, 2006). The giant mole-rat is a social species, weighing 250–600 g (males) and 200–350 g (females), which inhabits grassland and Miombo woodland areas in the Democratic Republic of Congo, Angola and Zambia (Kawalika & Burda, 2007; Bennett and Burda in Happold, 2013).

Nine giant mole-rats (195.8 ± 51.8 g), eight Cape mole-rats (156.4 ± 56.9 g), six Cape dune mole-rats (620.5 ± 142.8 g) and eight silvery mole-rats (196.4 ± 33.2 g), all non-breeding adults, were used in this study. Individuals of the silvery mole-rat and the giant mole-rat were born or kept for at least one year in captivity in the Department of Zoology (University of South Bohemia, Czech Republic). In contrast, individuals of the Cape dune mole-rat and the Cape mole-rat were captured about four months before the experiment and kept in the mole-rat research facility at the Department of Zoology and Entomology (University of Pretoria, South Africa).

Families of the giant mole-rat were housed in large terraria with a home box and plastic tubes to supply enrichment. Horticultural peat was used as bedding and tissue paper as nest material. In the case of the silvery mole-rat, individuals were housed in a system of interconnected Perspex tunnels with two home boxes. These animals were fed *ad libitum* with carrots,

potatoes and apples. Inside the breeding room, T_a was $25 \pm 1^\circ\text{C}$, relative humidity was $45 \pm 5\%$ and light regime was 12L:12D. Similarly, Cape-dune mole-rats and Cape mole-rats were kept in a temperature-controlled room at T_a of 23°C , with a 12L:12D photoperiod and air humidity between $50 \pm 10\%$. These species were housed in plastic boxes with wood shavings used as a bedding. The Cape mole-rats were fed *ad libitum* with sweet potatoes, while the Cape dune mole-rats received sweet potatoes, carrots and fresh grass.

Physiological parameters

Flow-through respirometry (Lighton, 2008) was used to estimate metabolic rate. In all cases, oxygen consumption ($\dot{V}\text{O}_2$) was measured using a computerized positive pressure open-flow respirometry system (Sable Systems, Las Vegas, NV, USA), following the similar procedure in both laboratories. Animals were individually placed in chambers differing in size depending on the species (between 1.8 and 6 L) that received air between 450 mL min^{-1} and 5 L min^{-1} from, either a FoxBox integrated flowmeter (silvery mole-rat and giant mole-rat) or an Omega mass-flow regulator (Cape mole-rat and Cape dune mole-rat), respectively (standard temperature and pressure corrected). Air passed through a CO_2 -absorbent (Soda lime, Sigma-Aldrich, St. Louis, MO, USA) and a water scrubber (Drierite, W.A. Hammond Drierite Co., Xenia, OH, USA) before going through the chamber. Excurrent air from the chamber was subsampled at $100 \pm 10 \text{ mL min}^{-1}$ and was passed through Soda lime and Drierite, before being analyzed by the O_2 analyzer (FoxBox or FC-10, Sable Systems, Las Vegas, NV, USA). The gas analyzer was calibrated using standard procedures. First, zero offset of the instrument was set using nitrogen (at least 99.995% purity). Second, span calibration (20.95%) was implemented using dry, CO_2 -free air. The metabolic chamber was kept at a stable temperature that was monitored using the thermal probe of a digital thermometer inserted in the chamber. Individual activity was observed through the transparent Perspex lid of the chamber during recording. All experimental trials were conducted at T_a of 26°C (Cape mole-rat and Cape-dune mole-rat) or 30°C (silvery mole-rat and giant mole-rat), which are T_a s within the TNZ of each species (Lovegrove, 1986, 1987; Bennett, Jarvis & Cotterilli, 1993; Zelová *et al.*, 2007). Metabolic rate (i.e., rates of O_2 consumption) was calculated using equation 4a of Withers (1977),

$$\dot{V}\text{O}_2 = V(\text{FiO}_2 - \text{FeO}_2/1 - \text{FiO}_2)$$

where V is the flow rate through the system, and FiO_2 and FeO_2 are the fractional O_2 concentrations of the incurrent and excurrent air, respectively (FiO_2 was 0.2095). Body mass (M) was measured before each experimental set using an electronic balance, and body temperature (T_b) using a RET-2 temperature probe (Physitemp Instruments LLC., Clifton, NJ, USA; tip diameter 3.2 mm, inserted at least 2 cm in the rectum) connected to Thermalert TH-8 (Physitemp Instruments LLC., Clifton, NJ, USA) or by an intraperitoneally injected PIT tags (<1 g, LifeChip[®] with Bio-Thermo[™] Technology; Destron Fearing

Corp., Dallas, TX, USA). A veterinarian injected the tags under anesthesia (Isofurane) three months before the experiment. The tags were calibrated by the manufacturer and were read using a Global Pocker Reader EX (Destron Fearing Corp., Dallas, TX, USA).

Resting metabolic rate (RMR) and maximal non-shivering thermogenesis (NST_{max}) were measured using the protocol described by Luna *et al.*, (2012). Briefly, after a period of habituation inside the chamber (~30 min), metabolic rate was recorded at rest for at least 1 h (Resting). This time allowed for obtaining a period of 5–10 min of the lowest steady-state of O_2 consumption. Once the RMR value was obtained, the animals were injected intramuscularly with a saline solution and their metabolic rate was recorded for 30–50 min. The same protocol was used 2 days later. After a period of habituation, similar to those used in control trial previous to saline injection, individuals were injected with norepinephrine (NE) instead of the saline solution (the same volume as in the saline control). According to Feist & Rosenmann (1976), the oxygen consumption response to NE occurs 10 min after its injection and lasts approximately 5–10 min. Doses of NE were estimated according to Wunder & Gettinger (1996),

$$\text{NE (mg kg}^{-1}\text{) doses} = 2.53 M^{-0.4}$$

The maximum metabolic rate obtained during a 10 min-steady-state after the injection of NE was considered to be the NST_{max} , which includes both RMR and thermoregulatory NST. Thus, NST is NST_{max} minus RMR. Wet thermal conductance (C_{wet}) was calculated as $C_{\text{wet}} = \text{MR} (T_b - T_a)^{-1}$, where MR is metabolic rate; this is because T_b does not equal T_a when the MR curve is extrapolated to the T_a axis (see McNab, 1980).

Conventional data analysis and statistics

Data are presented as mean \pm SD. We compared the mass-specific metabolic rate (msMR, $\text{mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$) for each species measured during resting, after saline injection and after NE injection and resting metabolic rate. Tests were performed by single-tailed pairwise Wilcoxon rank sum test, and we adjusted the P -levels by the Benjamini–Hochberg method to account for false discovery rate (Benjamini & Hochberg, 1995). The same test was performed with T_b ($^\circ\text{C}$) and C_{wet} ($\text{mL O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$). Tests were performed in R (R Core Team, 2018) inside RStudio (R Studio Team, 2015). To compare NST of bathyergids to that of surface-dwelling rodents, we re-organized the allometric equation that relates body mass to NST as proposed by Rodríguez-Serrano & Bozinovic (2009). We carried out this correction given that certain species of bathyergids and other fossorial rodents were included in the original dataset, such as *Cryptomys* spp., *Ctenomys* spp. and *S. cyanus*.

Phylogenetic framework and analysis

To evaluate whether phylogenetic relationships affect mass-specific NST ($\text{NST}_{\text{max}} - \text{RMR}$) in bathyergids, we first constructed a topological phylogenetic tree that included the bathyergids in which NST was measured (see Table 2). The tree

was constructed by subsetting the mammal tree from vertlife.org website (<https://vertlife.org/phylosubsets/>; Upham, Esselstyn & Jetz, 2019). We downloaded 100 pseudo-posterior distribution trees, which were used to produce a single maximum clade credibility (MCC) tree using the TreeAnnotator utility (Rambaut & Drummond, 2018) in BEAST (Drummond & Rambaut, 2007). A sub-species of *C. hottentotus* was added manually to the MCC tree using Mesquite (Maddison & Maddison, 2019).

The amount of phylogenetic signal for NST and M were estimated using Pagel's lambda parameter (λ , Pagel, 1999). Pagel's λ is a scaling parameter weighting the influence of the phylogeny for explaining the observed phenotypic data among species due to their shared evolutionary history. Pagel's λ was chosen because the Brownian Motion (BM) model of trait evolution is better than other parameters that evaluate phylogenetic signal (Münkemüller *et al.*, 2012). For Pagel's λ , values close to zero indicate independence from the phylogeny, whereas values equal to one indicate that the phylogeny is the most important factor explaining variation among species than that expected under BM. To evaluate whether Pagel's λ was significantly different than zero, a likelihood ratio test for the observed λ was used. This analysis was performed with the function 'phylosig' R package 'phytools' (Revell, 2012). To test whether a NST challenge differs between solitary and social species, phylogenetic ANOVA (Garland *et al.*, 1993) was implemented, under 1000 simulations, with 'phytools' package (Revell, 2012). The relationship between NST and M or environmental variables was evaluated using a phylogenetic generalized linear model (PGLS). After transforming NST and M (logarithm), PGLS analysis was run with 'caper' (Orme *et al.*, 2018) and 'ape' (Paradis, Claude & Strimmer, 2004) packages. As T_a can impose selective pressures on thermogenic capacity (Rezende, Bozinovic & Garland, 2004), we evaluated the effect of environmental T_a on NST within bathyergids. To characterize thermal environment of the area in which each bathyergid species inhabits, we obtained mean annual air temperature (T_{med}), minimum air temperature of the coldest month (T_{min}), maximum air temperature of the warmest month (T_{max}) and annual air temperature range (TAR: difference between T_{max} and T_{min}), for different sources. For *B. suillus* and *G. capensis*, the climatic data of the collecting sites were downloaded from the WorldClim database (<http://www.worldclim.org/>), using Diva-Gis (<http://www.diva-gis.org/>). For the remaining species, climatic data were obtained from Luna *et al.*, (2017). Climatic data for each species are shown in Table S1.

To ensure the regression model provided the best fit among all candidate models (possible models by the combination of M , T_{med} , T_{max} , T_{min} and TAR), we used the second-order Akaike Information Criterion (AIC_c; Burnham & Anderson, 2001, 2002). The best model of the set is that with the lowest AIC_c value. We also measured the Akaike weight (ω_i) to estimate the probability that any given model is the best fit compared with the other models. Models with Δ_i values less than 2 are considered to be informative and be essentially as good as the best model (Burnham, Anderson & Huyvaert, 2011). All phylogenetic analyses were developed in R (R Core Team, 2018) inside RStudio (R Studio Team, 2015).

Results

Metabolic rate increased in all species after NE injection ($P < 0.05$, see Fig. 1). Maxima were equivalent to $292 \pm 95\%$, $252 \pm 40\%$, $168 \pm 43\%$ and $158 \pm 43\%$ of the thermoneutral RMR for *G. capensis*, *F. mechowii*, *H. argenteocinereus* and *B. suillus*, respectively. Metabolic rate did not increase after saline injection in any of the study species ($P > 0.38$, see Fig. 1).

Body temperature after NE injection significantly increased in *G. capensis*, *F. mechowii*, *H. argenteocinereus*, but not in *B. suillus*. The T_b s were 1.4 ± 1.0 °C, 0.5 ± 0.8 °C and 0.7 ± 0.6 °C higher than the T_b during rest for *G. capensis*, *F. mechowii* and *H. argenteocinereus*, respectively, and not different in *B. suillus* (0.0 ± 0.3 °C). The T_b increased significantly after saline injection in all species ($P < 0.05$, Table 1).

The C_{wet} after NE injection increased in all species ($P < 0.05$, see Table 2). The increases were to $227 \pm 59\%$, $224 \pm 33\%$, $145 \pm 32\%$ and $158 \pm 39\%$ of the resting C_{wet} in *G. capensis*, *F. mechowii*, *H. argenteocinereus* and *B. suillus*, respectively. The C_{wet} after saline injection did not increase in any species ($P > 0.22$, Table 1).

Mass-specific NST of *G. capensis*, *F. mechowii*, *H. argenteocinereus* and *B. suillus* were 43.5%, 40.1%, 17.3% and 13.5%, respectively (Table 2), compared to the reconstructed allometric equation for surface-dwelling rodents [$\log \text{msNST} (\text{mL O}_2 \text{ g}^{-1} \text{ h}^{-1}) = -0.34 \log M + 1.17$ ($R^2 = 0.22$, $F_{1,31} = 9.09$, $P < 0.05$; modified from Rodríguez-Serrano & Bozinovic, 2009)].

To assess mass-specific NST differences among bathyergids, we first evaluated if there was an influence of phylogeny. No phylogenetic signal was observed in mass-specific NST in bathyergids, since λ was close to 0 ($\lambda < 0.001$, $P = 0.99$). No differences in mass-specific NST were observed between social and solitary bathyergids (Phylogenetic ANOVA, $F_{1,7} = 8.21$, $P = 0.07$). When we analyzed if mass-specific NST is influenced by body mass and/or any climatic variables, such as T_{med} , T_{max} , T_{min} and TAR, Akaike criterion for the bathyergid dataset showed that only three of 27 models were informative (i.e., $\Delta_i < 2$). The best model is those that only include $\log M$. The best-fit phylogenetic regression between \log mass-specific NST and $\log M$ for bathyergids was as follows: $\log \text{NST} (\text{mL O}_2 \text{ g}^{-1} \text{ h}^{-1}) = -0.86 \log M + 1.70$ ($R^2 = 0.79$, $F_{1,6} = 25.64$, $P = 0.002$, Fig. 2). When we ordered the different models on the basis of their Δ_i values and with their corresponding ω_i values, the second and third best-fit models were that which included $\log M + T_{min}$ and $\log M + \text{TAR}$, as predictors, respectively. The probability of models (ω_i) is presented in Table 3. In Table S2, all models are presented that explain NST in bathyergids in relation to body mass and environmental variables.

Discussion

The increase in metabolic rate after NE injection determines the amount of heat that an individual can produce by NST (Cannon & Nedergaard, 2011). This is the first study that has measured NST capacity of solitary bathyergid species, and our

Table 1 Body temperature (T_b), metabolic rate (MR) and wet thermal conductance (C_{wet}) in four mole-rat species during resting, after saline and noradrenaline (NE) injection

Species	T_b (°C)				MR (mL O ₂ h ⁻¹)				C_{wet} (mL O ₂ g ⁻¹ h ⁻¹ °C ⁻¹)			
	Resting		NE		Resting		NE		Resting		NE	
	Saline	NE	Saline	NE	Saline	NE	Saline	NE	Saline	NE	Saline	NE
<i>Georchus capensis</i>	35.0 ± 0.4	35.5 ± 0.6**	36.5 ± 0.8**	36.5 ± 0.8**	98.6 ± 38.9	97.5 ± 35.1	259.9 ± 70.6**	259.9 ± 70.6**	124.6 ± 21.1	114.8 ± 18.7	278.0 ± 60.1**	278.0 ± 60.1**
<i>Fukomys mechowii</i>	34.5 ± 0.4	34.4 ± 0.6**	35.0 ± 0.6*	35.0 ± 0.6*	118.1 ± 15.6	114.6 ± 30.6	297.9 ± 63.7**	297.9 ± 63.7**	142.1 ± 35.3	135.4 ± 25.2	309.8 ± 44.4**	309.8 ± 44.4**
<i>Heliophobius argenteocinereus</i>	34.7 ± 0.7	34.7 ± 0.6**	35.4 ± 0.3*	35.4 ± 0.3*	127.3 ± 29.4	133.3 ± 22.4	206.7 ± 43.2**	206.7 ± 43.2**	137.8 ± 12.8	147.2 ± 26.1	198.1 ± 36.2**	198.1 ± 36.2**
<i>Bathyergus suillus</i>	35.4 ± 0.3	35.5 ± 0.2*	35.4 ± 0.2	35.4 ± 0.2	231 ± 47.1	249.8 ± 49.8	365.4 ± 111.2*	365.4 ± 111.2*	69.4 ± 8.3	74.0 ± 7.3	109.1 ± 27.8**	109.1 ± 27.8**

Differences to resting state were tested by single-tailed pairwise Wilcoxon rank sum test, P -levels adjusted by Benjamini-Hochberg method. Statistically significant differences are marked by asterisks.

* $P < 0.05$.

** $P < 0.01$.

data, together with published information, allowed us to analyze NST variation in bathyergid species varying in their sociality. After NE injection, metabolic rates of the solitary *H. argenteocinereus*, *G. capensis* and *B. suillus* showed fractional increases of 1.7, 2.8 and 1.6, respectively, whereas the corresponding value in the social *F. mechowii* was 2.7. In the latter species, the observed fractional increase is within the range of other social bathyergids, which varied from a minimum of 1.9 in *F. damarensis* (Hislop & Buffenstein, 1994) to as high as 4.5 in the *Cryptomys h. pretoriae* (Haim & Fairall, 1986).

As proposed earlier, increases in NST affect thermal balance in mole-rats (Haim & Fairall, 1986; Woodley & Buffenstein, 2002; Broekman *et al.*, 2006). Hislop & Buffenstein (1994) evaluated NST in the naked mole-rat *H. glaber* and the Damaraland mole-rat *F. damarensis* and established its effect on T_b . Non-shivering capacity was higher in *H. glaber*, but the increase in T_b was lower than in *F. damarensis*. Whereas the T_b of *H. glaber* increased $\sim 2.8^\circ\text{C}$ after NE injection, the T_b of *F. damarensis* increased by $\sim 4.1^\circ\text{C}$. This difference may be associated with the relatively higher heat loss in *H. glaber* due to the absence of fur and its relatively high surface to volume ratio (Hislop & Buffenstein, 1994). Moreover, Broekman *et al.*, (2006) found that high-elevation populations of *C. h. mahali* exhibited higher NST and higher T_b compared to their low-altitude counterparts ($3.23 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ vs. $2.16 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ and 38.2°C vs. 36.1°C , respectively). These differences were ascribed to the higher thermal conductance observed in low-elevation individuals. Thus, T_b might vary according to the relationship between the amount of heat produced by NST and the amount of heat loss, hence C_{wet} . In our case, for example, the T_b of *H. argenteocinereus* and *F. mechowii* are similar following NE injection ($35.4 \pm 0.3^\circ\text{C}$ vs. $35.0 \pm 0.2^\circ\text{C}$, respectively), but *H. argenteocinereus* showed a lower C_{wet} , due to their denser and longer fur compared to the giant mole-rat (Šumbera *et al.*, 2007b). Thus, the balance between lower MR and lower C_{wet} after NE injection in *H. argenteocinereus* compared to *F. mechowii* could determine the similarity in T_b between both species. The relatively low NST of *H. argenteocinereus* might be sufficient to maintain stable T_b , without any further increase in energy expenditure compared to *F. mechowii* having less insulating fur. This finding is in agreement with Šumbera *et al.*, (2007b), which evaluated surface temperature (T_s). Using IR thermography, it was established that the differences in T_s between *H. argenteocinereus* and *F. mechowii* were related to the insulation capacity of their fur at low T_s . In well furred body regions, such as the head and trunk, *H. argenteocinereus* shows a T_s $\sim 3^\circ\text{C}$ lower than in *F. mechowii* (Šumbera *et al.*, 2007b). Although no NST estimation was performed, Boyles *et al.*, (2012) proposed that the longer and denser fur of *C. h. pretoriae* might be more important for thermoregulation of this species living in the colder environment than that of the Mashona mole-rat *Fukomys darlingi* living in a warmer climate. Furthermore, McGowan *et al.*, (2020) evaluated T_b and T_s of different social bathyergids and suggested that species living in colder environments prioritize increasing heat generation and insulating against heat loss, such as is the case of Natal mole-rat

Table 2 Mean body mass (M), non-shivering thermogenesis (NST), and predicted NST (%) in African mole-rats

Species	M (g)	NST ^a (mL O ₂ h ⁻¹)	% ^b		Reference
<i>Bathyergus suillus</i>	620	134	13.5	Solitary	This study
<i>Cryptomys hottentotus pretoriae</i>	102	177	57.1	Social	Haim & Fairall, 1986
<i>Cryptomys hottentotus mahali</i>	57	122	58.2	Social	Broekman <i>et al.</i> , 2006
<i>Fukomys damarensis</i>	123	141	40.5	Social	Hislop & Buffenstein, 1994
<i>Fukomys mechowii</i>	197	191	40.1	Social	This study
<i>Georychus capensis</i>	156	161	43.5	Solitary	This study
<i>Heliophobius argenteocinereus</i>	196	82	17.3	Solitary	This study
<i>Heterocephalus glaber</i>	36	55	35.2	Social	Woodley & Buffenstein, 2002

^aNST calculated as NST_{max}-RMR

^bPercentage of predicted NST (NST_{max}-RMR) by curve for rodents: NST (mL O₂ g⁻¹h⁻¹) = 14.7 M^{0.34} (modified from Rodríguez-Serrano & Bozinovic, 2009).

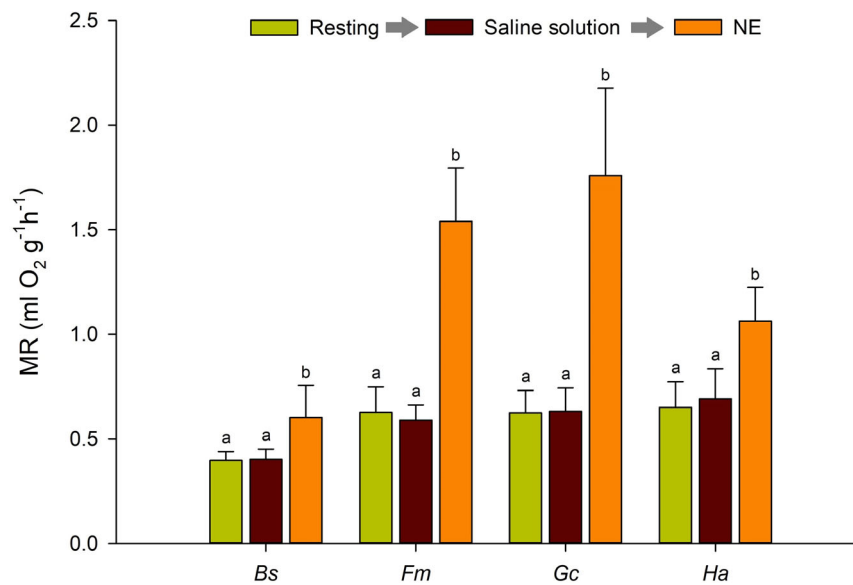


Figure 1 Metabolic rates of *Bathyergus suillus* (Bs), *Fukomys mechowii* (Fm), *Georychus capensis* (Gc) and *Heliophobius argenteocinereus* (Ha) in different experimental conditions. Experimental design: 1. Metabolic rate at resting (Resting). 2. Metabolic rate after injection of saline solution (Saline solution). 3. Metabolic rate after injection of norepinephrine injection (NE). See Materials and methods for details. Small numbers refer to comparisons among experimental conditions for each species.

C. h. natalensis. Thus, bathyergid species living in cold conditions might show a tendency to follow an energy conservation strategy, taking advantage of the insulative fur (i.e., low C_{wet}) rather than increasing heat production.

However, NST has been proposed to be quite plastic, since it can vary according to long-term exposures to low T_a s (Lovegrove, 2005) so it may exhibit seasonal acclimatization in environments with pronounced seasonal fluctuations of ambient temperature. In *H. argenteocinereus* and *F. mechowii*, dispersal occurs at the end of the rainy season and at the beginning of the cold and dry season when temperature is relatively low (Šumbera, Burda & Chitaukali, 2003; Kawalika & Burda, 2007; Šumbera *et al.*, 2007a). Although data are very anecdotal, it appears that individuals of the social *F. mechowii* disperse on their own (Lövy, Šklíba & Šumbera, 2013). Thus, the higher NST values can be established to withstand longer

periods without a partner and lack of thermoregulatory savings coming from huddling with conspecifics. A higher potential for NST could be advantageous for social species, because recent findings suggest individuals of social subterranean rodent species have higher body heat loss compared to their solitary counterparts (Vejmělka *et al.*, 2021). Even if isolated individuals of social species meet a partner and breed, social thermoregulation might not be efficient if the family is small. This may explain the differences between NST in this species and the solitary *H. argenteocinereus* occupying the same habitat and climatic conditions. On the other hand, the solitary *G. capensis* showed higher values of NST very similar to those in *F. mechowii*. However, this may be explained by its occurrence in the southernmost part of the African continent where it faces cooler temperate climates. Lower values of NST (and no increase in T_b after NE injection) in *B. suillus* from the

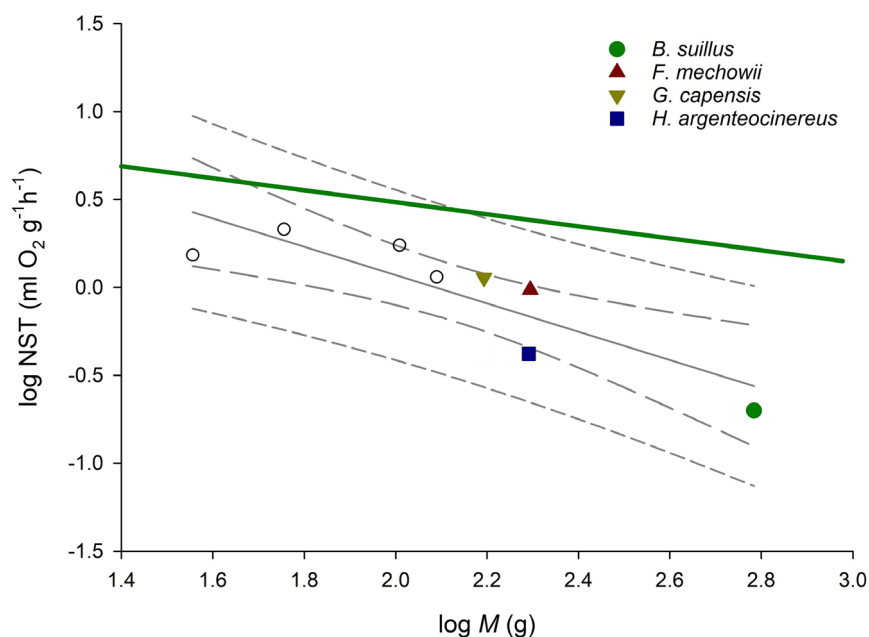


Figure 2 Regression between body mass ($\log M$) and mass-specific non-shivering thermogenesis ($\log NST$) in bathyergid species. Inner long-dash lines represent the 95% confidence interval. Outer short-dash lines represent the 95% prediction interval. Green solid line represents allometric equation for surface-dwelling rodents (modified from Rodríguez-Serrano & Bozinovic, 2009).

Table 3 Akaike Information Criterion (AICc) values, AICc differences (Δ_i), and Akaike weights (ω_i) for the informative models for bathyergids. Body mass (M) and environmental variables (T_{\min} or TAR) are the predictive variables

Models (bathyergids)	AICc	R^2	Intercept	$\log M$	T_{med}	T_{max}	T_{min}	TAR	Δ_i	ω_i
$\log \text{msNST} \sim \log M$	-2.48	0.79	1.70	-0.86	0	0	0	0	0	0.41
$\log \text{msNST} \sim \log M + T_{\min}$	-2.17	0.86	1.73	-0.77	0	0	-0.02	0	0.31	0.34
$\log \text{msNST} \sim \log M + \text{TAR}$	-0.80	0.83	1.10	-0.79	0	0	0	0.02	1.68	0.17

T_{\min} , minimum temperature of the coldest month; T_{\max} , maximum temperature of the warmest month; TAR, annual temperature range (difference between T_{\max} and T_{\min}).

same region, living in some areas even sympatrically with *G. capensis*, could be explained by the large body mass and thus more favorable surface area to volume ratio in this the largest of subterranean rodents. Nevertheless, to uncover effects of social, ecological and other factors on NST thermogenesis in bathyergids, analyses of more species occurring in different climates and of differing degree of sociality will be necessary. Unfortunately, the total number of solitary species of mole-rats within the family is very low, precluding any conclusive statistical test allowing identification of such factors. For such purposes, extending the analysis to other fossorial rodents or even mammals generally could be the way forward as to how best to test intriguing relationships between sociality and magnitude of the NST.

In endotherms, the effect of body mass on thermogenic variables is well-understood (McNab, 2002; Withers *et al.*, 2016). In rodents, body mass alone explains generally around 70% of the variation in resting metabolism and total thermogenic capacity (Wunder & Gettinger, 1996; Rezende *et al.*, 2004). Similarly, thermogenic and energetic characteristics of

bathyergids are mainly associated with body mass (Šumbera, 2019). Indeed, our study demonstrates that around 82% of the variation of NST between bathyergids can be explained by body mass. Interestingly, the second-best model includes body mass and one of climatic parameters (i.e., T_{\min}), together explaining ~86% of variation of NST (Table 3). In a largescale analysis, Rodríguez-Serrano & Bozinovic (2009) found that NST of rodents with different lifestyles is evolutionarily labile (i.e., there is a lack of phylogenetic signal) and negatively correlated with T_{\min} and T_{\max} . Our results indicate that rodents living in cold climates exhibit higher NSTs than species from warm climates. In general, for bathyergids, variation of T_a inside burrows is mainly determined by T_a at the surface (Šumbera, 2019). In this sense, the burrow T_a which is associated with surface T_a was proposed to be the main factor to explain NST variations between the populations of *C. h. mahali* living at different altitudes (Broekman *et al.*, 2006). Our finding is also supported by finding in another strictly subterranean rodent, the blind mole-rat *S. ehrenbergi* in which the magnitude of NST was

found to be related to monthly T_a variation between different geographic areas (Haim *et al.*, 1984).

The fact that the NST is partially explained by T_{\min} , or by the annual range of T_a , suggests climate has played a role in the evolution of NST variation in bathyergids. For this group, NST appears to be lower than expected compared to the allometric equation for surface-dwelling rodents. In evolutionary terms, the relatively low NST responses of bathyergids compared to surface rodents may be determined by the attenuation of T_a inside burrows. Thus, despite the effect of T_a ; the daily and seasonal stability of T_a inside burrows may be advantageous, resulting in a relatively low NST compared to surface-dwelling rodents. For example, at similar body mass, NST of the mountain ground squirrel *Xerus princeps* (1.29 mL O₂ g⁻¹ h⁻¹, 627 g; Haim, Skinner & Robinson, 1987) is 5.9 times higher than the NST of the Cape dune mole-rat in our study (0.22 mL O₂ g⁻¹ h⁻¹, 620 g). Moreover, the interplay between NST and thermal insulation (i.e., C_{wet}) may ensure T_b maintenance in these conditions. Notably, the negative slope of the regression of bathyergids is steeper than this relationship proposed for surface-dwelling rodents (see Results).

On the other hand, burrows can impose restrictions on the total thermogenic capacity, due to external factors other than T_a . It has been suggested that burrows are characterized by atmospheres with low O₂ (hypoxic) and high CO₂ (hypercapnic) concentrations (Nevo, 1999). Thus, hypoxic and hypercapnic atmosphere of burrows were considered as important factors in the evolution of subterranean species, since these could be found to be higher or lower under different environmental conditions or in the nests as well as when burrowing. As proposed by Luna *et al.*, (2012) for the fossorial Tala's tuco-tucos, NST may well have evolved under particular atmospheric conditions in burrowing animals and this could well be responsible for the low NST in these rodents compared to surface rodents. However, available data on gas composition from the burrows of African mole-rats have revealed the burrow partial pressures of O₂ and CO₂ do not deviate much from the aboveground atmosphere (Roper *et al.*, 2001; Šumbera *et al.*, 2004; Holtze *et al.*, 2018). Moreover, burrow atmosphere effect on NST could be unlikely for bathyergids, since only extremely severe hypoxia produces changes in ventilatory responses, energy metabolism and T_b variations (Zhang & Pamerter, 2019; Devereaux & Pamerter, 2020; Ivy *et al.*, 2020).

For future studies, it would be interesting to study shivering thermogenesis (ST). Total thermogenic capacity can vary according to the extent of its components (Wunder & Gettinger, 1996). In some fossorial species of the genus *Ctenomys*, NST is not the exclusive heat-producing mechanism. Depending on the species, NST and ST in *Ctenomys* contribute differently to total thermogenic capacity, being in some cases the total thermogenic capacity determined by ST alone (Luna *et al.*, 2019). Although no specific work has been performed to evaluate ST in bathyergids, it has been reported that some individuals of solitary species have been shown to shiver at lower T_{as} (Lovegrove, 1987; Marhold & Nagel, 1995; Šumbera *et al.*, 2007a; Begall *et al.*, 2015). Therefore, the presence of ST as a heat-producing mechanism in bathyergids

needs to be evaluated. In this sense, the occurrence and importance of ST in solitary and social bathyergids deserve further attention.

In conclusion, functional NST is evident in *H. argenteocinereus*, *G. capensis*, *B. suillus* and *F. mechowii*. Contrary to our expectations, NST was not found to be different between species with different social organizations, suggesting that the potential use of social thermoregulation has no effect in lowering the energy required for thermogenesis, at least for NST. At interspecific level, body mass is the most important factor influencing NST variation among different species of bathyergids. However, as with other rodents, their NST is affected also by the climate (T_{\min}), even though there is attenuation of T_a inside burrows. The T_{as} inside burrows is relatively stable and below the TNZ, so an interplay between thermal insulation (i.e., C_{wet}), behavior and NST may ensure T_b maintenance in these conditions. The relative stability of T_a of burrows can affect total thermogenic capacity, hence the NST variation in bathyergids, since NST is relatively lower than surface-dwelling rodents of similar body mass. As a consequence, body mass and T_a impose a high selective pressure on NST in this group of rodents, requiring further studies to evaluate ST as a contribution to total thermogenic capacity. Thus, to fully understand variation in NST among bathyergids, analyses of more species living under different climatic conditions and differing in their degree of sociality are necessary. Furthermore, the relationship between sociality and the magnitude of NST can be investigated using this type of analysis in other families of fossorial rodents such as the Geomyidae and Ctenomyidae.

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Ethics statement

The experimental procedures in the Czech Republic were approved by the University of South Bohemia Animal Welfare committee and Ministry of Education, Youth and Sports of the Czech Republic (Permission no. MSMT-26065/2014-16), while the procedures in South Africa were approved by University of Pretoria Animal Ethics Committee (Permission no. EC069-16). A capture research permit was issued by Western Cape Nature Conservation (Permission no. AAA041-00169-0056).

Conflict of interest

Authors declare no potential conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Body mass (M), mass-specific non-shivering thermogenesis (NST) and climatic data for bathyergids species.

Table S2. Models that explain NST in bathyergids with relation to log-transformed body mass ($\log M$) and environmental variables (T_{med} : mean annual temperature; T_{min} : minimum temperature of the coldest month; T_{max} : maximum temperature of the warmest month; TAR: annual temperature range as the difference between T_{max} and T_{min}).