The hypoxia tolerance of eight related African mole-rat species rivals that of naked mole-rats, despite divergent ventilatory and metabolic strategies in severe hypoxia

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Abstract

Aims: Burrowing mammals tend to be more hypoxia tolerant than non-burrowing mammals and rely less on increases in ventilation and more on decreases in metabolic rate to tolerate hypoxia. Naked mole-rats (*Heterocephalus glaber*, NMRs), eusocial mammals that live in large colonies, are among the most hypoxia-tolerant mammals, and rely almost solely on decreases in metabolism with little change in ventilation during hypoxia. We hypothesized that the remarkable hypoxia tolerance of NMRs is an evolutionarily conserved trait derived from repeated exposure to severe hypoxia owing to their burrow environment and eusocial colony organization.

Methods: We used whole-body plethysmography and indirect calorimetry to measure the hypoxic ventilatory and metabolic responses of eight mole-rat species closely related to the NMR.

Results: We found that all eight species examined had a strong tolerance to hypoxia, with most species tolerating 3 kPa O_2, *Heliophobius emini* tolerating 2 kPa O_2 and *Bathyergus suillus* tolerating 5 kPa O_2. All species examined employed a combination of increases in ventilation and decreases in metabolism in hypoxia, a response midway between that of the NMR and that of other fossorial species (larger ventilatory responses, lesser reductions in metabolism). We found that eusociality is not fundamental to the physiological response to hypoxia of NMRs as *Fukomys damarensis*, another eusocial species, was among this group.

Conclusions: Our data suggest that, while the NMR is unique in the pattern of their physiological response to hypoxia, eight closely related mole-rat species share the ability to tolerate hypoxia like the current "hypoxia-tolerant champion," the NMR.

Keywords: chemosensitivity; control of breathing; fossorial; hypoxic metabolic response; hypoxic ventilatory response; thermoregulation

1 INTRODUCTION

The use of underground burrows is widespread in mammals. Burrows offer protection from inclement environmental conditions and also predators.[1] However, confined burrow spaces can present challenges for their occupants in the form of extreme atmospheric conditions. In social species where numerous animals live within the confined space of the nest, and there is poor gas diffusion through the surrounding soils, the conditions faced (intermittent acute (or chronic) hypoxic and hypercarbic conditions[1-3]) can be severe. However, reports of the composition of gases, and thus the severity of hypoxia and hypercarbia, in burrows of fossorial species vary greatly, from 20 to 6 kPa O_2 and 1 to 9.5 kPa CO_2.[1, 4-9] The timing and location of burrow measurements presumably contribute to this variability, as well as other factors, such as burrow structure, soil type, number of occupants and degree of fossoriality.[1, 10]

The response to hypoxia in mammals may consist of increases in ventilation (to match O_2 supply to metabolic demand; the hypoxia ventilatory response), decreases in metabolic rate (to match metabolic demand to O_2 supply; the hypoxic metabolic response) or a combination of the two.[11-14] It has long been hypothesized that repeated exposure to relative hypoxia and hypercarbia compared with surface gas composition (ie normoxia and normocarbia) has shaped the homeostatic responses to these conditions in semi-fossorial and fully fossorial mammals,[1, 3] and that their greater reliance on metabolic depression and lesser reliance on ventilatory increases compared with non-fossorial mammals contributes to their greater tolerance to hypoxia. This is borne out in experimental evidence where non-fossorial mammals, which tend to be less tolerant to hypoxia, typically exhibit increases in total ventilation and little change to metabolic rate,[15] whereas fossorial and semi-fossorial mammals, which are typically more tolerant of hypoxia compared with non-fossorial mammals, exhibit a reduced ventilatory response in conjunction with various degrees of metabolic suppression.[15] Semi-fossorial species typically do not mount a hypoxic response until PO_2 has fallen to lower levels than observed for non-burrowing species, and there appears to be little variability in the ventilatory responses of fossorial and semi-fossorial mammals to hypoxia, as well as to hypercarbia. However, reports from fully fossorial species are sparse.

One of the few fully fossorial species examined, the naked mole-rat (NMR; *Heterocephalus glaber*), is eusocial, and is among the most hypoxia-tolerant mammals currently identified. NMRs are able to maintain consciousness and stay active even in severe hypoxia (<5% O_2), which are hallmarks of hypoxia tolerance.[16, 17] NMRs are able to withstand minutes of anoxia,[18] hours of 3 kPa O_2[19] and days in 8 kPa O_2,[20] where hypoxia tolerance is partially

mediated by depressions in metabolic rate, body temperature and behavioural activity with little change in ventilation,[17, 19-21] making them an exception with regard to the strategy by which semi-fossorial and fully fossorial species tolerate hypoxia. This strategy is thought to contribute to making the NMR a champion of hypoxia tolerance in mammals, an idea exemplified by the physiological response to hypoxia of the African Damaraland mole-rat (*Fukomys damarensis* [*F.d.*]), one of the other few fully fossorial, eusocial species examined. African Damaraland mole-rats are able to tolerate 5 kPa O_2 for short periods,[22] and their strategy resembles that of a non-fossorial species, in that a ventilatory response is mounted at 12 kPa inspired O_2 and no metabolic depression is observed.[22] These data suggest that (a) the strategy by which semi- and fully fossorial mammals tolerate hypoxia is conserved (although there have only been a few fully fossorial mammals examined), and that (b) NMRs are an exception in both their strategy and tolerance to hypoxia.

Given the differences between two of the fully fossorial mammals examined (NMRs and Damaraland mole-rats), we sought to determine the extent to which hypoxia tolerance and the phenotypic balance between the hypoxic ventilatory response and the hypoxic metabolic response observed in NMRs are evolutionarily conserved or the result of adaptive evolution by studying several closely related mole-rat species. We examined 8 closely related African mole-rat species that vary in their sociality (Figure 1, Table 1) using the whole-animal barometric method of plethysmography combined with indirect calorimetry to measure ventilation and metabolic rate, respectively. We exposed each species to 18, 12 (mild hypoxia), 9, 7 (moderate hypoxia), 5 and, where possible, 3 and 2 (severe hypoxia) kPa O_2 for 30 minutes each, and measured metabolic rate, ventilation and body temperature. We wished to determine the extent to which each species exhibited increases in ventilation, decreases in metabolism and body temperature and whether the patterns of change were associated with differences in hypoxia tolerance.

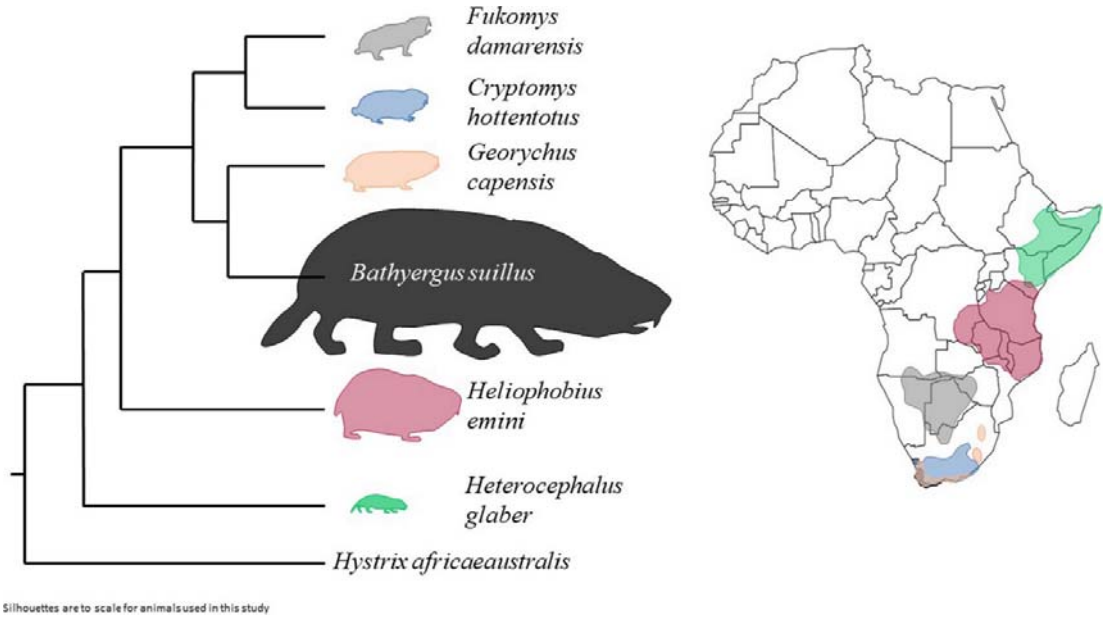

Figure 1. Phylogeny of mole-rat species used in this study. Adapted from Ref. [56, 57], distribution maps adapted from Ref. [58-63]

Table 1. Common names and characteristics of the eight mole-rat species used in this study

Species name	Abbreviated	Common name	Fossoriality	Sociality	Colony size	Aridity	Burrow gas composition	
							O_2 (%)	CO_2 (%)
Fukomys damarensis	*F.d.*	Damaraland mole-rat	Fossorial	Eusocial	12-41[25, 47]	Arid	20.4	0.4[9]
Cryptomys hottentotus mahali	*C.h.m.*	Mahali mole-rat	Fossorial	Social	9-20[48]	Humid	Unknown	Unknown
Cryptomys hottentotus pretoriae	*C.h.p.*	Highveld mole-rat	Fossorial	Social	6-12[49, 50]	Semi-arid	Unknown	Unknown
Cryptomys hottentotus hottentotus	*C.h.h.*	Common mole-rat	Fossorial	Social	5-14[47, 51, 52]	Arid	Unknown	Unknown
Cryptomys hottentotus natalensis	*C.h.n*.	Natal mole-rat	Fossorial	Social	7-16[53]	Humid	Unknown	Unknown
Georychus capensis	*G.c.*	Cape mole-rat	Fossorial	Solitary	1[54]	Humid	20.4	0.4[9]
Bathyergus suillus	*B.s.*	Cape dune mole-rat	Semi-fossorial	Solitary	1[54]	Semi-arid	Unknown	Unknown
Heliophobius emini	*H.e.*	Silvery mole-rat	Fossorial	Solitary	1	Humid	19.8 ± 0.4[55]	Unknown
Heterocephalus glaber	NMR	Naked mole-rat	Fossorial	Eusocial	80-250[25]	Semi-arid	20.5 ± 0.3	0.17 ± 0.09[7]

Note

Aridity based on Ref. [31].

2 RESULTS

2.1 African mole-rats mount a ventilatory response to acute hypoxia

All mole-rat species increased ventilation in response to acute hypoxia challenges, however, the contribution of breathing frequency and tidal volume to this increase differed (Figure 2, Tables 2 and 3). In most mole-rats, total ventilation did not significantly increase under moderate hypoxia (>5 kPa O_2), whereas *Cryptomys hottentotus hottentotus* (*C.h.h.*), *F.d.* and *Bathyergus suillus* (*B.s.*) did not increase total ventilation until the most severe levels of hypoxia were administered (≤3 kPa O_2) (Figure 2A, Table 2). These effects are reflected by significant main effects of inspired PO_2 for all mole-rat species (Tables 2 and 3). The change in total ventilation from resting conditions in severe hypoxia was an ~ 2-fold increase and was similar among mole-rat species, with the exception of *Georychus capensis* (*G.c.*), which exhibited an ~ 5-fold increase in the most severe level of hypoxia (3 kPa O_2; Figure 2A, Table 3). In most of the social mole-rat species (*F.d.*, *Cryptomys hottentotus mahali* [*C.h.m.*], *Cryptomys hottentotus pretoriae* [*C.h.p.*] and *Cryptomys hottentotus natalensis* [*C.h.n.*]), breathing frequency significantly increased in moderate hypoxia, but not in severe hypoxia, with *C.h.h.* not significantly increasing breathing frequency at any level of hypoxia tested (Figure 2B, Table 2). The fall in breathing frequency in the most severe levels of hypoxia was likely caused by significant increases in tidal volume in all social mole-rat species, including *C.h.h*. Thus, hypoxic ventilation still increased compared with normoxic ventilation (Figure 2C, Table 2). In the solitary mole-rat species (*B.s.*, *Heliophobius emini* [*H.e.*], *G.c.*), breathing frequency was observed to significantly increase in *B.s.* and *G.c.* in moderate and severe hypoxia, whereas *H.e.* did not significantly alter breathing frequency (Figure 2B, Table 2). *H.e.* and *G.c.* were observed to significantly increase tidal volume in severe hypoxia, whereas *B.s.* did not exhibit a tidal volume response to hypoxia challenge (Figure 2C, Table 2).

Table 2. Ventilatory responses to acute hypoxia of eight mole-rat species with the *F*- and *P*-values for the main effect of PO_2

PO_2 (kPa)	*B.s.*	*H.e.*	*G.c.*	*F.d.*	*C.h.m.*	*C.h.p.*	*C.h.n.*	*C.h.h.*
Total ventilation (mL kg^{-1} min^{-1})								
18	511.8 ± 107.0	586.1 ± 109.4	469.4 ± 67.6	690.5 ± 73.7	694.7 ± 102.3	637.5 ± 84.6	650.9 ± 69.7	877.2 ± 109.1
12	502.6 ± 104.9	517.9 ± 100.5	501.7 ± 54.5	726.6 ± 97.6	732.5 ± 84.5	831.4 ± 85.5	689.2 ± 52.9	901.2 ± 216.7
9	491.1 ± 75.1	660.9 ± 159.7	687.8 ± 72.0	703.5 ± 92.1	924.0 ± 170.5	904.1 ± 113.9	734.1 ± 68.4	793.3 ± 106.8
7	556.4 ± 86.7	751.3 ± 131.8	721.9 ± 53.0	900.9 ± 104.8	963.3 ± 121.2	811.8 ± 79.0	850.2 ± 52.0	921.0 ± 128.4
5	652.5 ± 105.3[a]	1178.9 ± 191.6[a]	986.2 ± 95.0[a]	945.1 ± 144.3	1130.7 ± 133.7[a]	986.2 ± 127.9	1166.9 ± 124.8[a]	974.3 ± 163.1
3		1206.1 ± 67.8[a]	2135.0 ± 239.6[a]	1410.7 ± 255.9[a]	981.5 ± 197.7	1274.6 ± 241.8[a]	1348.4 ± 140.0[a]	1342.6 ± 149.7[a]
2		1226.8 ± 126.4[a]						
Main effect of PO_2								
F	3.076	32.27	38.98	4.544	3.581	3.551	12.00	9.553
P	.028	<.001	<.001	.002	.009	.009	<.001	<.001
Breathing frequency (breaths min^{-1})								
18	65.05 ± 11.9	108.3 ± 12.0	43.29 ± 4.12	79.02 ± 8.27	69.65 ± 4.58	64.96 ± 6.48	64.49 ± 6.58	93.49 ± 8.49
12	72.64 ± 11.1	102.9 ± 10.1	54.54 ± 4.77	77.11 ± 6.66	76.20 ± 3.55	90.53 ± 5.74	70.02 ± 3.89	91.41 ± 8.51
9	81.71 ± 8.82[a]	109.5 ± 10.9	72.14 ± 5.97[a]	79.64 ± 8.79	101.68 ± 9.35[a]	91.90 ± 7.41	70.24 ± 5.92	88.00 ± 8.94
7	89.19 ± 7.95[a]	116.1 ± 12.8	73.10 ± 6.93[c]	96.13 ± 5.77	90.40 ± 4.88[a]	78.81 ± 6.33	76.41 ± 4.62	78.33 ± 8.94
5	91.72 ± 7.48[a]	136.8 ± 19.2	76.61 ± 4.10[a]	98.56 ± 4.23	86.90 ± 4.84[a]	75.54 ± 4.62	86.11 ± 5.95[a]	78.00 ± 9.74
3		129.8 ± 9.36	104.3 ± 6.84[a]	88.55 ± 4.36	69.54 ± 5.61	81.37 ± 10.69	82.31 ± 4.89[a]	107.3 ± 16.9
2		98.76 ± 5.80						
Main effect of PO_2								
F	8.890	1.417	19.37	2.778	7.324	2.151	3.345	5.461
P	<.001	.249	<.001	.030	<.001	.079	.012	<.001
Tidal volume (mL kg^{-1})								
18	7.66 ± 0.43	6.18 ± 1.13	11.32 ± 1.72	8.95 ± 0.63	9.59 ± 1.03	9.97 ± 1.06	10.44 ± 0.85	8.11 ± 0.94
12	7.05 ± 1.34	6.29 ± 1.12	9.41 ± 0.93	9.38 ± 0.98	9.43 ± 0.86	9.33 ± 0.88	9.87 ± 0.57	8.03 ± 1.34
9	6.07 ± 0.76	7.64 ± 1.98	9.54 ± 0.73	8.33 ± 0.49	8.82 ± 1.06	9.79 ± 0.88	10.59 ± 0.66	7.81 ± 1.16
7	6.03 ± 0.51	7.66 ± 1.40	10.38 ± 0.96	7.91 ± 0.58	10.46 ± 1.02	10.46 ± 0.96	11.30 ± 0.67	10.49 ± 1.51
5	7.08 ± 0.85	10.31 ± 1.81[a]	13.14 ± 1.33	9.28 ± 1.14	13.13 ± 1.47[a]	12.71 ± 1.09	13.47 ± 0.82[a]	10.44 ± 1.24
3		10.51 ± 1.08[a]	20.84 ± 2.19[a]	15.37 ± 2.07[a]	13.83 ± 2.44[a]	14.85 ± 1.32[a]	16.44 ± 1.36[a]	15.28 ± 2.54[a]

PO_2 (kPa)	*B.s.*	*H.e.*	*G.c.*	*F.d.*	*C.h.m.*	*C.h.p.*	*C.h.n.*	*C.h.h.*
2		14.26 ± 1.75[a]						
Main effect of PO_2								
F	1.627	20.64	10.18	5.056	5.119	8.033	10.93	12.76
P	.189	<.001	<.001	.001	<.001	<.001	<.001	<.001

Abbreviation: PO_2, partial pressure of O_2.

[a] Represents a significant difference from resting/normoxic conditions using Holm-Sidak post-tests. n = 10 *Bathyergus suillus* (*B.s.*), n = 5 *Heliophobius emini* (*H.e.*), n = 10 *Georychus capensis* (*G.c.*), n = 10 *Fukomys damarensis* (*F.d.*), n = 10 *Cryptomys hottentotus mahali* (*C.h.m.*), n = 9 *Cryptomys hottentotus pretoriae* (*C.h.p.*), n = 10 *Cryptomys hottentotus natalensis* (*C.h.n.*), n = 10 *Cryptomys hottentotus hottentotus* (*C.h.h.*).

Table 3. Main effect of PO_2 *F*- and *P*-values of mole-rat ventilatory and metabolic responses to acute hypoxia challenge

Species	Fold change in total ventilation		Fold change in breathing frequency		Fold change in tidal volume		Oxygen consumption rate		Ventilatory equivalent for O_2		Pulmonary O_2 extraction		Body temperature	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
B.s.	4.099	.008	10.10	<.001	1.746	.161	2.835	.038	5.965	<.001	10.89	<.001	89.43	<.001
H.e.	16.59	<.001	1.369	.267	12.39	<.001	6.431	.004	7.937	.002	1.468	.275	102.7	<.001
G.c.	23.15	<.001	17.29	<.001	7.256	<.001	10.09	<.001	26.87	<.001	3.318	.020	126.3	<.001
F.d.	4.933	.001	3.588	.009	5.331	<.001	4.299	.005	7.158	<.001	2.389	.061	123.4	<.001
C.h.m.	5.926	<.001	8.315	<.001	6.644	<.001	2.682	.056	6.240	.002	2.224	.097	123.5	<.001
C.h.p.	3.958	.005	2.663	.036	5.992	<.001	5.245	.003	17.08	<.001	3.731	.016	38.65	<.001
C.h.n.	10.74	<.001	3.570	.008	9.575	<.001	7.674	<.001	9.230	<.001	3.278	.018	163.7	<.001
C.h.h.	12.51	<.001	4.35	.003	12.14	<.001	12.00	<.001	18.15	<.001	1.454	.23		

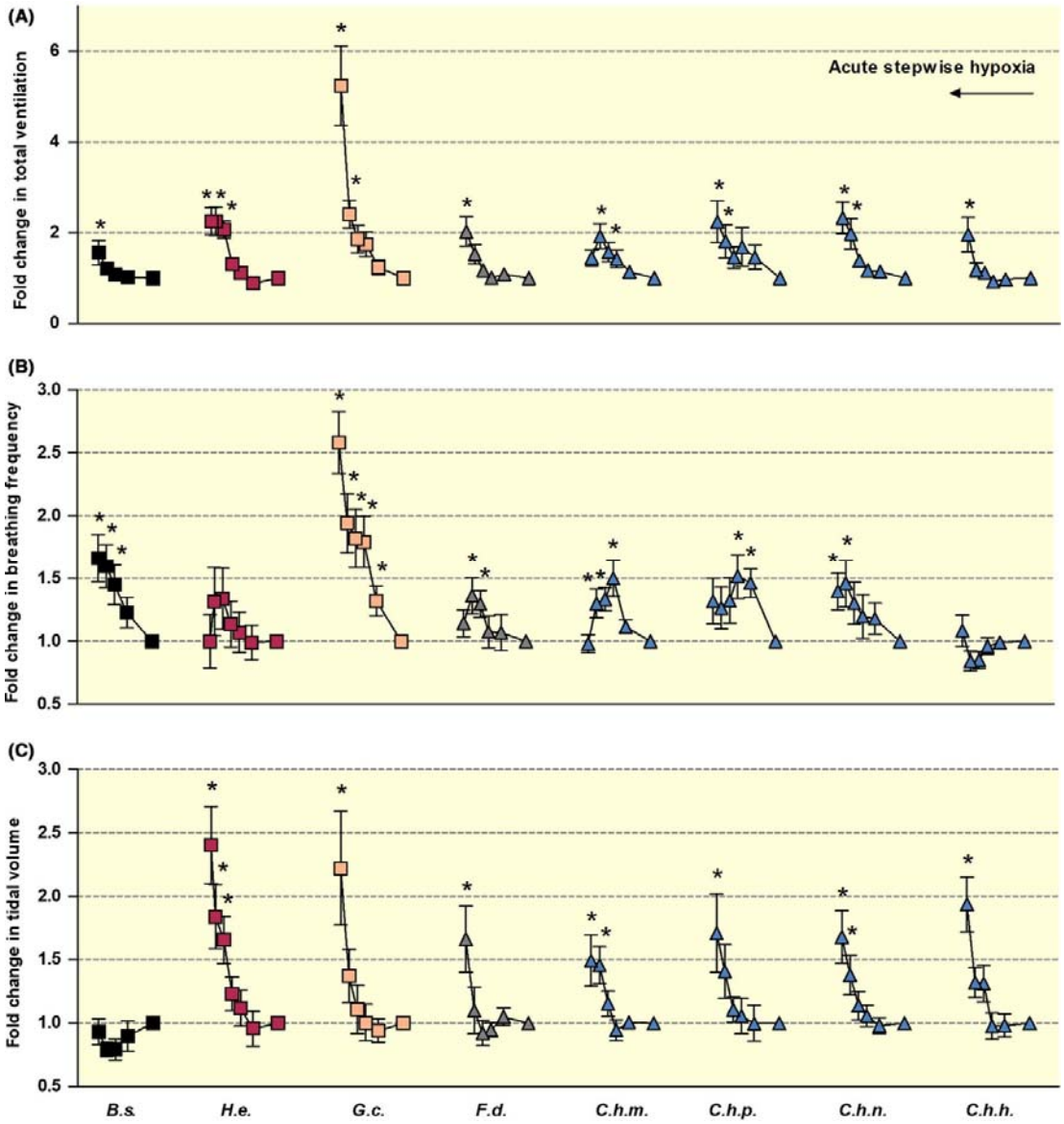

Figure 2. Mole-rats exhibit blunted fold changes in ventilatory responses to acute hypoxia challenge (A), with increases in breathing frequency (B) occurring in modest levels of hypoxia, and tidal volume (C) increasing in the most severe levels of hypoxia. Responses to acute hypoxia for each species are shown from right to left for stepwise reductions in inspired O_2 tension (PO_2): 18, 12, 9, 7, 5 (deepest level for *B.s.*), 3 and 2 (only *H.e.* tested at this PO_2) kPa O_2. *Represents a significant difference from resting/normoxic conditions using Holm-Sidak post-tests; squares denote solitary species, triangles denote social species. n = 10 *Bathyergus suillus* (*B.s.*), n = 5 *Heliophobius emini* (*H.e.*), n = 10 *Georychus capensis* (*G.c.*), n = 10 *Fukomys damarensis* (*F.d.*), n = 10 *Cryptomys hottentotus mahali* (*C.h.m.*), n = 9 *Cryptomys hottentotus pretoriae* (*C.h.p.*), n = 10 *Cryptomys hottentotus natalensis* (*C.h.n.*), n = 10 *Cryptomys hottentotus hottentotus* (*C.h.h.*)

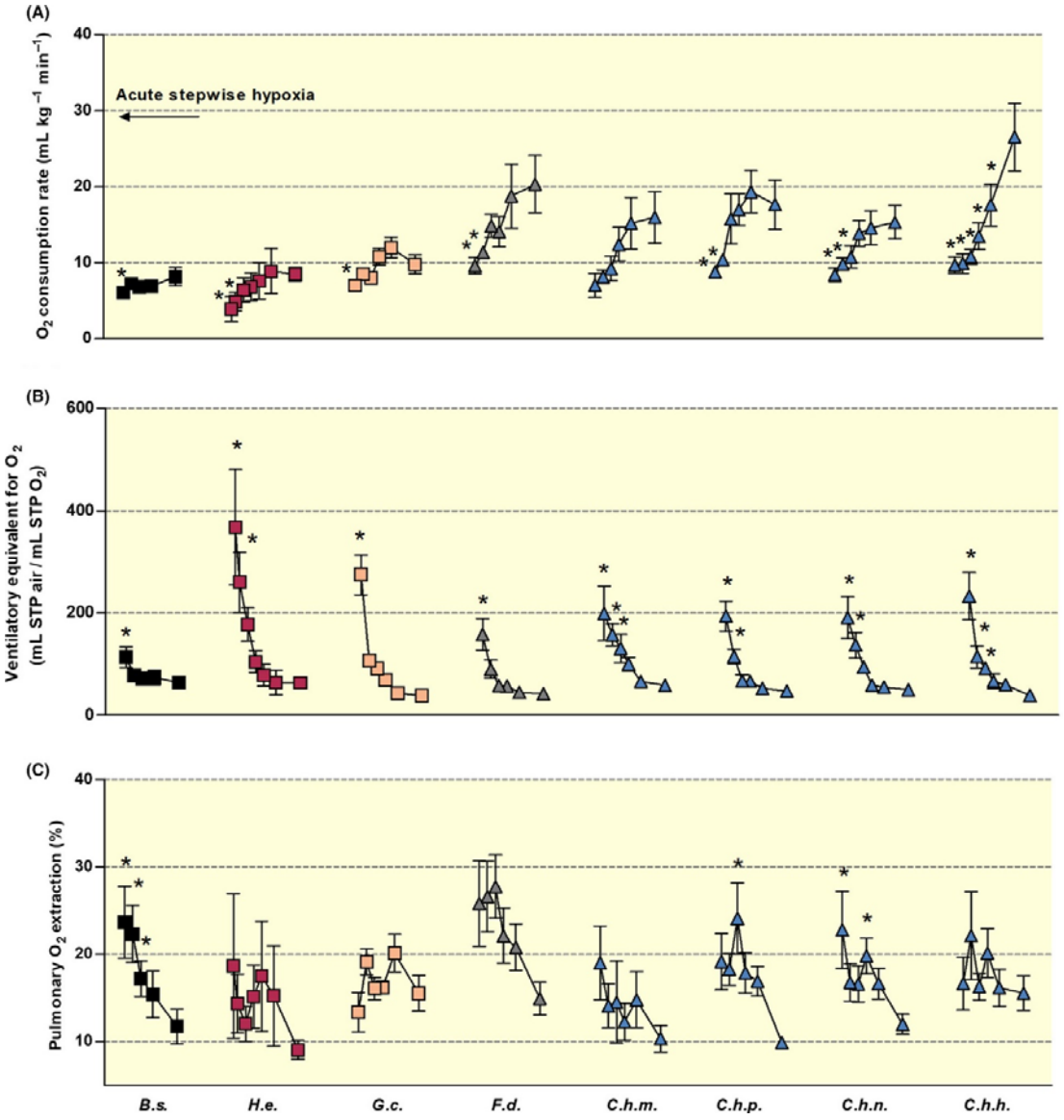

Figure 3. All mole-rats depressed O_2 consumption (A) in the most severe levels of hypoxia, resulting in increases in ventilatory equivalent for O_2 (B), with variable influences on pulmonary O_2 extraction (C). Responses to acute hypoxia for each species is shown from right to left for stepwise reductions in inspired O_2 tension (PO_2): 18, 12, 9, 7, 5 (deepest level for *B.s.*), 3 and 2 (only *H.e.* tested at this PO_2) kPa O_2. *Represents a significant difference from resting/normoxic conditions using Holm-Sidak post-tests; squares denote solitary species, triangles denote social species. N as for Figure 2

2.2 Mole-rats depress metabolic rate in response to hypoxia

O_2 consumption rate ($\dot{V}O_2$) and CO_2 production rate ($\dot{V}CO_2$) declined significantly in all mole-rats during the hypoxic challenge (Figure 3A, Table 4), reflected by a significant main effect of PO_2 in all species (Tables 3 and 4). The respiratory exchange ratio was not significantly influenced by hypoxia in any species (Table 4). The social species (*F.d.*, *Ch.m.*, *C.h.p.*, *C.h.n.*) tended to show a greater degree of metabolic depression in hypoxia compared with the solitary species (*B.s.*, *H.e.*, *G.c.*), but they also had higher resting $\dot{V}O_2$ in

Table 4. Metabolic responses to acute hypoxia of eight mole-rat species with the F- and P-values for the main effect of PO_2

PO_2 (kPa)	*B.s.*	*H.e.*	*G.c.*	*F.d.*	*C.h.m.*	*C.h.p.*	*C.h.n.*	*C.h.h.*
Ventilatory equivalent for CO_2 (mL min^{-1}/mL CO_2 min^{-1})								
18	67.65 ± 13.45	56.62 ± 15.56	38.50 ± 2.39	44.36 ± 4.03	54.88 ± 6.35	48.49 ± 8.76	55.93 ± 6.05	45.29 ± 4.84
12	74.89 ± 12.74	71.16 ± 13.88	46.66 ± 4.86	56.74 ± 4.49	60.19 ± 9.72	58.68 ± 8.21	60.97 ± 7.02	75.47 ± 12.16
9	81.36 ± 10.94	87.00 ± 34.67	77.59 ± 4.27	63.40 ± 7.06	99.19 ± 6.24	79.38 ± 10.47	72.94 ± 5.79	77.66 ± 8.18
7	87.87 ± 12.96	124.2 ± 57.50	89.65 ± 10.16[a]	73.11 ± 11.22	134.1 ± 14.65[a]	87.60 ± 7.96	103.8 ± 6.23	109.8 ± 11.94
5	117.4 ± 21.46	178.1 ± 26.05	117.62 ± 9.94[a]	110.9 ± 19.36[a]	185.2 ± 25.71[a]	120.5 ± 17.72[a]	146.1 ± 23.18[a]	129.6 ± 20.24[a]
3		252.6 ± 69.24	248.5 ± 27.28[a]	175.8 ± 26.34[a]	208.1 ± 30.66[a]	177.9 ± 24.40[a]	200.3 ± 33.33[a]	245.8 ± 56.82[a]
2		303.6 ± 103.0[a]						
Main effect of PO_2								
F	1.682	4.722	45.655	11.637	8.974	10.883	10.713	9.708
P	.171	.009	<.001	<.001	<.001	<.001	<.001	<.001
CO_2 production (mL kg^{-1} min^{-1})								
18	7.41 ± 0.79	10.20 ± 1.05	9.34 ± 0.81	17.98 ± 2.82	15.85 ± 2.32	16.76 ± 3.39	13.37 ± 1.47	22.03 ± 2.86
12	6.61 ± 0.63	7.18 ± 1.03[a]	11.01 ± 0.76[a]	13.45 ± 1.95[a]	15.42 ± 2.02	16.71 ± 2.22	12.32 ± 0.92	13.51 ± 1.84[a]
9	6.06 ± 0.51	7.71 ± 1.61[a]	9.60 ± 0.87	12.01 ± 1.27[a]	11.71 ± 2.34	14.06 ± 1.50	10.93 ± 1.09[a]	10.25 ± 0.74[a]
7	6.35 ± 0.53	7.08 ± 1.75[a]	8.31 ± 0.86[a]	11.55 ± 1.02[a]	8.22 ± 0.48[a]	11.09 ± 0.94[a]	9.09 ± 0.82[a]	8.99 ± 0.44[a]
5	5.76 ± 0.40	6.52 ± 1.07[a]	7.80 ± 0.78[a]	9.10 ± 0.64[a]	7.28 ± 0.54[a]	9.71 ± 0.55[a]	8.93 ± 0.59[a]	8.05 ± 0.34[a]
3		6.07 ± 1.39[a]	7.49 ± 0.44[a]	7.70 ± 0.46[a]	6.39 ± 0.59[a]	8.63 ± 0.50[a]	7.47 ± 0.64[a]	8.48 ± 0.69[a]
2		4.07 ± 0.76[a]						
Main effect of PO_2								
F	2.542	8.741	19.091	6.344	5.769	5.258	13.74	13.85
P	.056	.001	<.001	<001	.002	.003	<.001	<.001
Respiratory exchange ratio								
18	0.96 ± 0.06	1.09 ± 0.12	0.99 ± 0.11	0.93 ± 0.08	0.99 ± 0.05	0.99 ± 0.05	0.90 ± 0.07	0.87 ± 0.06
12	0.97 ± 0.04	0.86 ± 0.17	0.93 ± 0.04	0.78 ± 0.07	1.07 ± 0.14	0.89 ± 0.03	0.91 ± 0.07	0.84 ± 0.10

9	0.91 ± 0.04	0.75 ± 0.43	0.90 ± 0.04	0.89 ± 0.06	0.99 ± 0.10	0.85 ± 0.03	0.81 ± 0.05	0.83 ± 0.10
7	0.90 ± 0.04	0.79 ± 0.17	1.04 ± 0.07	0.80 ± 0.06	0.95 ± 0.10	0.78 ± 0.09a	0.90 ± 0.08	0.86 ± 0.05
5	0.96 ± 0.05	0.82 ± 0.04	0.92 ± 0.03	0.81 ± 0.05	0.95 ± 0.10	0.93 ± 0.06	0.92 ± 0.03	0.90 ± 0.10
3		0.83 ± 0.06	1.09 ± 0.08	0.88 ± 0.11	0.96 ± 0.09	0.99 ± 0.03	0.92 ± 0.07	0.92 ± 0.09
2		0.93 ± 0.22						
Main effect of PO_2								
F	0.530	0.193	1.289	0.657	0.216	2.629	0.382	0.344
P	.714	.973	.301	.659	.952	.049	.858	.882

Abbreviation: PO_2, partial pressure of O_2.

[a] Represents a significant difference from resting/normoxic conditions using Holm-Sidak post-tests. n = 10 *Bathyergus suillus* (*B.s.*), n = 5 *Heliophobius emini* (*H.e.*), n = 10 *Georychus capensis* (*G.c.*), n = 10 *Fukomys damarensis* (*F.d.*), n = 10 *Cryptomys hottentotus mahali* (*C.h.m.*), n = 9 *Cryptomys hottentotus pretoriae* (*C.h.p.*), n = 10 *Cryptomys hottentotus natalensis* (*C.h.n.*), n = 10 *Cryptomys hottentotus hottentotus* (*C.h.h.*).

normoxia (Figure 3A, Table 4). The fall in $\dot{V}O_2$ and increase in total ventilation in the most severe levels of hypoxia resulted in significant increases in ventilatory equivalent for O_2 and CO_2 across all species (Figure 3B, Tables 3 and 4). *B.s.* minimally increased their ventilatory equivalent for O_2 and CO_2 as a result of modest changes in ventilation, $\dot{V}O_2$ and $\dot{V}CO_2$. This was countered by significant increases in pulmonary O_2 extraction in the severe levels of hypoxia (Figure 3C, Table 3). *C.h.p.* and *C.h.n.* also significantly increased pulmonary O_2 extraction, but this was in combination with much larger increases in ventilation, $\dot{V}O_2$ and the ventilatory equivalent for O_2.

2.3 Body temperature falls in response to hypoxia challenge in all mole-rat species

Body temperature declined significantly in all mole-rats with acute hypoxia (Figure 4), reflected by a significant main effect of PO_2 in all species (Table 3). Body temperature declined ~2-6°C in response to acute hypoxia challenge, with *B.s.* exhibiting a more modest decline (~2°C) (Figure 4, Table 3). This depression brought mole-rat body temperature close to ambient temperature (~28°C), with variation in body temperature decline appearing to be associated with concomitant and comparable variation in $\dot{V}O_2$ depression (Figures 3A and 4, Table 3).

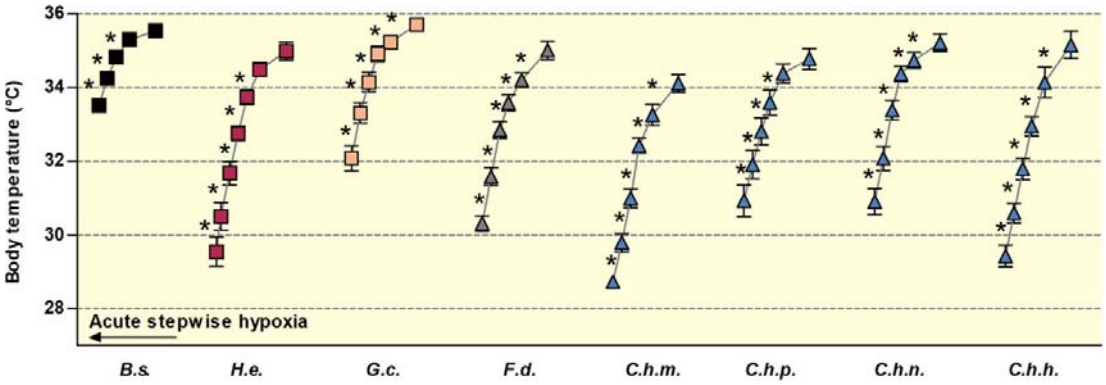

Figure 4. Body temperature was depressed in all mole-rat species during hypoxia challenge. Responses to acute hypoxia for each species is shown from right to left for stepwise reductions in inspired O_2 tension (PO_2): 18, 12, 9, 7, 5 (deepest level for *B.s.*), 3 and 2 (only *H.e.* tested at this PO_2) kPa O_2. *Represents a significant difference from resting/normoxic conditions using Holm-Sidak post-tests; squares denote solitary species, triangles denote social species. N as is for Figure 2

2.4 Blood properties are not significantly altered after 3 hours of hypoxia in mole-rat species

Most blood properties did not change after 3 hours of hypoxia in our mole-rat species (Table 5). All blood parameters measured in this study were not significantly altered after 3 hours of hypoxia in solitary mole-rat species, with only *G.c.* exhibiting a significant increase in pH with hypoxia exposure (t_8 = 2.661, P = .029). Increases in haematocrit (Hct) only occurred in the *Cryptomys* species (*C.h.m.* (t_8 = 5.595, P = .001), *C.h.p.* (t_8 = −2.473, P = .039) and *C.h.h.* (t_8 = −7.158, P < .001)) (Table 4). Hct was not significantly altered after 3 hours of hypoxia in the solitary mole-rat species. Only *C.h.p.* also increased haemoglobin (Hb) concentration

Table 5. Blood properties of mole-rats sampled after 3 hours of normoxia or hypoxia using an iStat and manual determination of haematocrit (Hct) and haemoglobin (Hb) concentration

Parameter	*Bathyergus suillus*		*Heliophobius emini*	*Georychus capensis*		*Cryptomys hottentotus mahali*		*Cryptomys hottentotus pretoria*		*Cryptomys hottentotus hottentotus*	
	Nx (n = 4)	**Hx (n = 3)**	**Nx (n = 3)**	**Nx (n = 5)**	**Hx (n = 5)**	**Nx (n = 5)**	**Hx (n = 5)**	**Nx (n = 5)**	**Hx (n = 5)**	**Nx (n = 5)**	**Hx (n = 5)**
pH	7.36 ± 0.03	7.39 ± 0.02	7.29 ± 0.06	7.28 ± 0.04	7.42 ± 0.03[a]	7.20 ± 0.07	6.28 ± 1.03	7.30 ± 0.05	7.38 ± 0.05	7.43 ± 0.05	7.33 ± 0.05
HCO_3^- (mmol L^{-1})	31.38 ± 2.17	25.00 ± 4.60	26.40 ± 2.20	19.84 ± 2.24	21.78 ± 1.07	11.15 ± 3.27	13.73 ± 4.50	23.95 ± 0.74	23.73 ± 2.72	22.33 ± 1.92	18.08 ± 1.09
Lactate (mg dL^{-1})	6.33 ± 0.32	7.47 ± 0.44	6.36 ± 2.77	10.90 ± 2.72	8.60 ± 0.55	10.88 ± 2.68	7.95 ± 3.06	7.88 ± 2.43	11.95 ± 1.98	4.09 ± 0.91	10.34 ± 1.48[a]
Hct (%, manual)	47.00 ± 1.06	48.79 ± 5.54	46.67 ± 4.40	48.92 ± 1.17	45.87 ± 2.53	36.73 ± 2.05	51.82 ± 1.86[a]	33.00 ± 1.91	51.59 ± 0.68[a]	34.73 ± 1.78	43.1 ± 2.88[a]
Hb (g dL^{-1}, manual)	16.67 ± 0.76	16.04 ± 4.66	11.19 ± 5.15	15.98 ± 1.32	16.57 ± 1.97	15.00 ± 1.60	13.10 ± 1.90	11.62 ± 1.14	18.46 ± 1.54[a]	14.47 ± 1.41	16.46 ± 1.29

Note

Values are mean ± SEM.

[a]Indicates a significant difference between normoxia- and hypoxia-exposed mole-rats within a species.

(t_8 = −3.575, P = .007), and *C.h.h.* was the only species to exhibit an increase blood lactate concentration (t_8 = −2.995, P = .024).

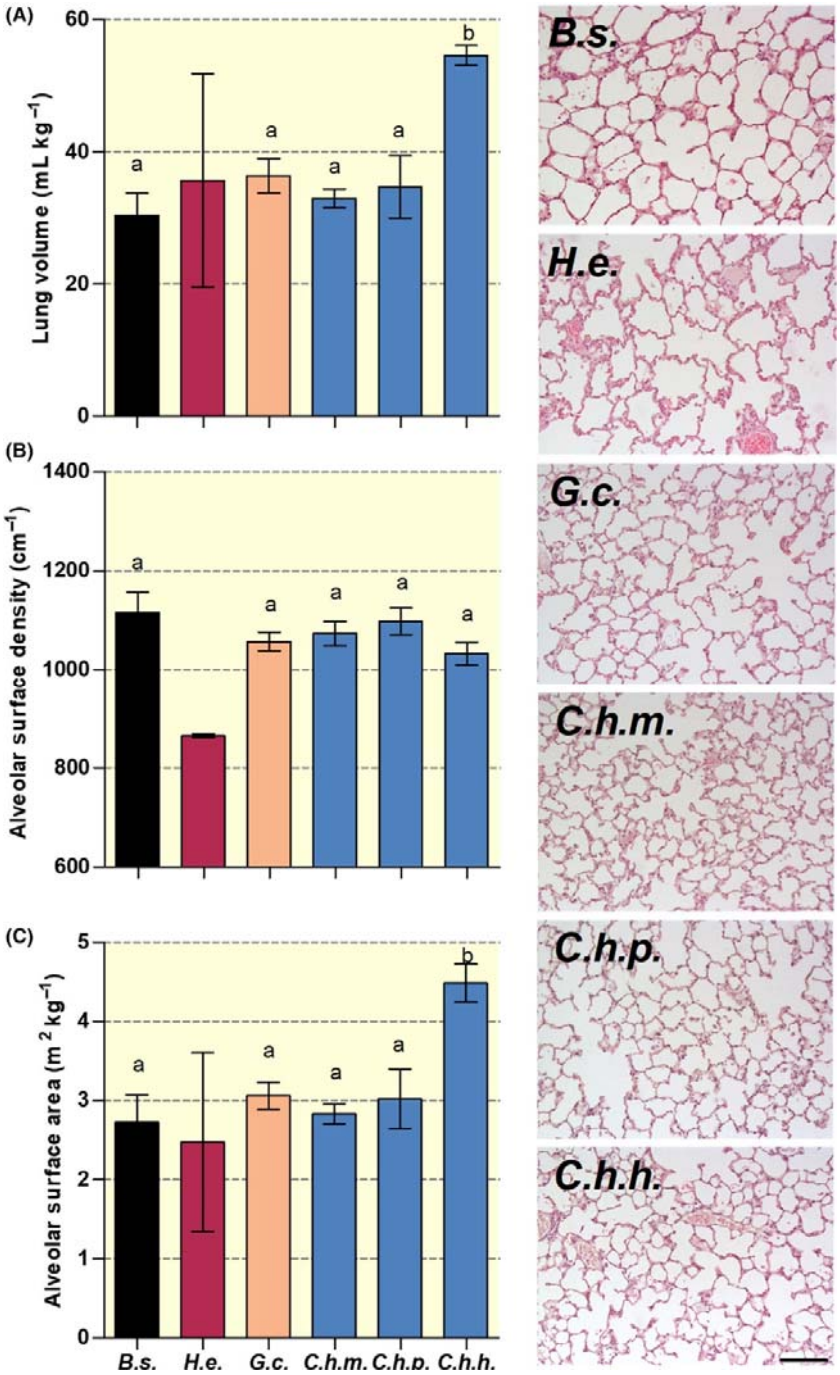

Figure 5. Lung volume (A), alveolar surface density (B) and alveolar surface area (C) were similar between all mole-rat species, with *C.h.h.* exhibiting larger lung volumes and alveolar surface area. Statistics were not conducted on *H.e.* owing to small sample size. Values are mean ± SEM, scale bar on representative images represents 50 µm. n = 7 *Bathyergus suillus* (*B.s.*), n = 2 *Heliophobius emini* (*H.e.*), n = 10 *Georychus capensis* (*G.c.*), n = 10 *Fukomys damarensis* (*F.d.*), n = 10 *Cryptomys hottentotus mahali* (*C.h.m.*), n = 9 *Cryptomys hottentotus pretoriae* (*C.h.p.*), n = 10, n = 10 *Cryptomys hottentotus hottentotus* (*C.h.h.*)

2.5 Lung volume and morphology are similar among mole-rat species

All mole-rats exhibited similar lung volumes and alveolar characteristics, except for *C.h.h.* (Figure 5). *C.h.h.* had a significantly larger lung volume than the other mole-rat species in this study, when corrected for body mass ($F_{4,17}$ = 8.201, P < .001; Figure 5A). Alveolar surface density was similar between all mole-rats, with *H.e.* having lower surface density (*H.e.* was not included in statistical analysis owing to a sample size of 2; $F_{4,17}$ = 1.524, P = .240; Figure 5B), but when adjusted for lung volume and body mass, only *C.h.h.* exhibited a significantly greater alveolar surface area ($F_{4,17}$ = 6.298, P = .003; Figure 5C).

3 DISCUSSION

Previous work has shown that the NMR is among the most hypoxia-tolerant mammals, responding to extreme hypoxia with no change in ventilation and large reductions in metabolism[17, 19, 20] (Figure 6). This is hypothesized to be the result of an evolutionary pressure exerted by low oxygen availability resulting from their fully fossorial and eusocial lifestyle,[1, 3] which could possibly be facilitated by a retention of neonatal traits.[23] Here, we examined whether (a) hypoxia tolerance and (b) a blunted ventilatory response combined with large reductions in metabolism are evolutionarily conserved in eight closely related South African mole-rat species, of which one (*F.d.*) species is also fully fossorial and is the only other known eusocial mammal. We found that while most were able to tolerate 3 kPa O_2, hypoxia-induced changes in ventilation and pulmonary gas exchange in mole-rat species from this study differed from that of NMRs from previous studies (Figure 6), in that all mole-rats investigated in this study ultimately exhibited a ventilatory response to progressive hypoxia and more modest falls in oxygen consumption. These findings do not appear to be associated with body size or sociality, suggesting that the absence of a ventilatory response to hypoxia in NMRs is an adaptation unique to this species. However, 6 of the mole-rat species examined in this study had larger decreases in O_2 consumption, smaller increases in ventilation frequency, and greater decreases in body temperature in acute hypoxia compared with non-fossorial species examined in previous studies (Figure 6). Taken together, these data suggest 1) either some degree of trait conservation between these species or a convergent evolution brought on by similar environmental pressures, and 2) that the reported species in this study, with the exception of *B.s.*, are as hypoxia tolerant as the current “champion” of hypoxia tolerance, the NMR.

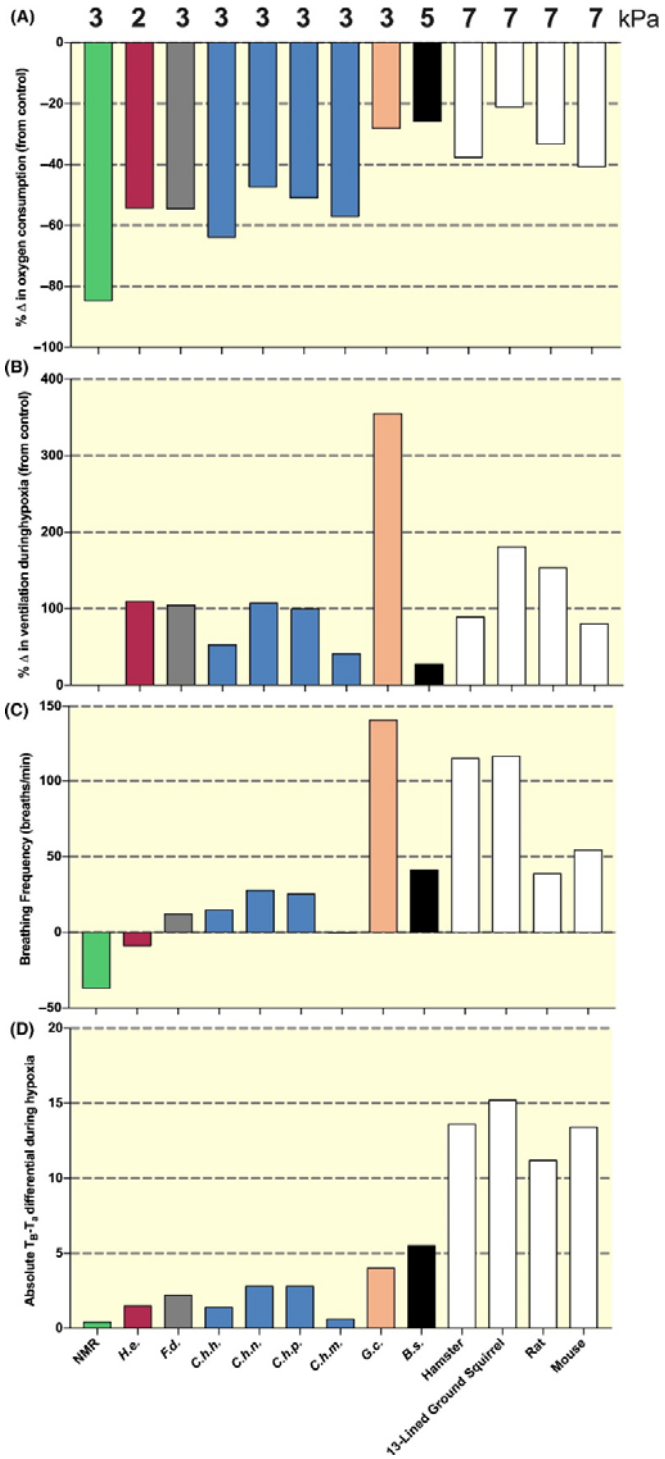

Figure 6. Summarizing graphic comparing the change in oxygen consumption (A), ventilation (B), breathing frequency (C) and body temperature differential (T_B-T_a) (D) among fully fossorial, eusocial mole-rats (*F.d.* [this study] and NMR), fully fossorial mole-rats (*H.e.*, *C.h.h.*, *C.h.n.*, *C.h.p.*, *C.h.m.*, *G.c.* and *B.s.* [this study]), semi-fossorial rodents (ground squirrel and hamster) and non-fossorial rodents (rats and mice) during the lowest level of hypoxia each species could tolerate (values in kPa above panel A). Naked mole-rat data adapted from Ref. [17, 19]. Ground squirrel, hamster, rat and mouse data adapted from Ref. [64]. Note that ventilation data for NMRs are unavailable at the reported level of hypoxia. NMR, Naked mole-rat

3.1 African mole-rats depress metabolism and body temperature, and increase ventilation in response to hypoxia

Semi-fossorial and fossorial mammals tend to have a greater tolerance for hypoxia compared with non-fossorial mammals.[15] Although a blunted ventilatory response to hypercarbia is common among burrowing mammals, the ventilatory response to hypoxia is more variable;[3, 15] the variability in the ventilatory response to acute hypoxia is apparent in this study as well as in previous studies (Figure 6). Specifically, species such as *C.h.h.*, *C.h.m.* and *B.s.* show little change in ventilation upon acute exposure to severe hypoxia, whereas other closely related species (*H.e.*, *F.d.*, *C.h.p.*, *C.h.n.*) increase ventilation under the same conditions, closely resembling the ventilatory responses from previous reports on fossorial species[15] (Figure 6). Curiously, *G.c.* has a ventilatory response that surpasses those of most other species. NMRs, on the other hand, exhibit little or no ventilatory response to hypoxia.[19, 20]

However, ventilation accounts for only part of the hypoxic response as many semi-fossorial and fossorial species employ a strategy that also includes decreases in metabolism.[15] NMRs combat low environmental O_2 with a large reduction in aerobic metabolism, thus matching O_2 demand to a depleted O_2 supply.[19, 20] The mole-rat species reported in this study, with the exception of *G.c.* and *B.s.,* exhibit decreases in metabolic rate that are greater than those observed in non-fossorial species from previous studies but not as large as that of the NMR (Figure 6). *B.s.* in this study exhibited only a modest fall in O_2 consumption and a very small increase in ventilation in hypoxia, thus sustaining its hypoxic metabolic rate by increasing pulmonary O_2 extraction (see below). *G.c.* also exhibited a modest fall in oxygen consumption but sustained its hypoxic metabolic rate with a large increase in ventilation.

It should be noted that the responses of the Damaraland mole-rat (*F.d.*) in this study were different than those reported previously. Zhang and Pamenter (2019) reported a fourfold increase in ventilation and no change in oxygen consumption in this species on exposure to 5 kPa O_2. At this level of hypoxia we report a modest (not yet significant) increase in ventilation and a roughly 50% decrease in O_2 consumption. The basis of this difference is not clear but might reflect the temperature at which each study was performed (30°C[22] vs 28°C [this study]), which may limit the scope for metabolic adaptation in hypoxia.

The net result is that we see a wide range of combinations of changes in ventilation, pulmonary O_2 extraction and O_2 consumption within mole-rats despite the fact that all are highly tolerant of severe hypoxia.

3.2 The pattern by which ventilation increases and pulmonary O_2 extraction is maintained differs among African mole-rat species

All mole-rats ultimately responded to acute hypoxia with increases in total ventilation. However, the contribution of increases in breathing frequency and tidal volume to this response differed between species. In all of the social mole-rat species (*F.d.*, *C.h.m.*, *C.h.p.*, *C.h.n.*), except *C.h.h.*, the contribution of breathing frequency and tidal volume was observed to switch as hypoxia became more severe, with frequency playing a greater role in moderate hypoxia and tidal volume a greater role in severe hypoxia (Figure 2B). This switch

between breathing frequency and tidal volume could occur to accommodate an increase in effective ventilation, thus reducing dead-space ventilation[3]; a strategy observed among other fossorial and non-fossorial species.[1] Conversely, *C.h.h.* and *H.e.* did not significantly alter breathing frequency with hypoxia, but instead relied on increases in tidal volume only to increase total ventilation. *G.c.* and *B.s.* also exhibited different ventilatory strategies compared with the other mole-rat species. *G.c.* increased both breathing frequency and tidal volume in severe hypoxia, whereas *B.s.* only increased breathing frequency and not tidal volume.

With the exception of *H.e.*, *G.c.* and *C.h.h.*, there was a trend for pulmonary O_2 extraction to increase in hypoxia (Figure 3C), although it was only significant in three of the species. These increases most likely reflect increases in pulmonary perfusion. Together, these responses suggest that there are multiple strategies for responding to acute hypoxia to maintain pulmonary O_2 extraction in closely related fossorial species. Interestingly, regardless of strategy, all species were still able to withstand more severe levels of hypoxia than non-fossorial species.[15]

3.3 Sociality does not influence hypoxia sensitivity

Consumption of ambient oxygen by large eusocial colonies has been suggested to be the driving mechanism for the evolution of hypoxia tolerance in NMRs.[24] Consistent with this, a similar level of hypoxia tolerance was also seen in *F.d.*, the one eusocial species in our study.[25] However, tolerance to a similar degree of hypoxia was also demonstrated for solitary (*B.s.*, *H.e.* and *G.c.*) and a range of social mole-rat genera (*Fukomys* and *Cryptomys* sp; see Figure 1 for average colony sizes), whereas one species (*H.e.*) was even more tolerant. Together, these findings suggest that life in large eusocial colonies may, at best, be only partially responsible for the tolerance to severe hypoxia observed in NMRs.

There also appears to be little influence of body size on hypoxia tolerance. Six species over a range from roughly 90 to 150 g were able to tolerate 3 kPa O_2. *B.s.*, the largest species (roughly 750 g), was only able to tolerate 5 kPa O_2, suggesting that hypoxia tolerance might be reduced in relatively large animals (see Figure 1 for scale), however, silvery mole-rats (*H.e.*), the second largest mole-rat studied, were able to withstand 2 kPa O_2, lending support to the suggestion that body size per se has little influence on hypoxia tolerance. This leads us to suggest that the tolerance to hypoxia that is apparently common among the species examined in this study, as well as in NMRs, was inherited from a common ancestor and differences in environmental pressures in the intervening time have shaped phenotype and thus contributed to the differences in strategy we see.

3.4 Blood properties do not influence hypoxia sensitivity

Many subterranean and high-altitude mammals exhibit alterations in the globin molecule for binding O_2, Hb concentration, and Hct, to enhance the movement of O_2.[26-29] Previous studies on Hb-O_2 binding affinities of African mole-rats showed no consistent patterns in Hct or blood and red cell 2,3-diphosphoglycerate (DPG) and Hb concentrations, or in intrinsic Hb-O_2 affinity and its sensitivity to pH and DPG that correlated with burrowing, sociality or soil type. However, the results did reveal a reduced effect of CO_2 on O_2 unloading that

would safeguard pulmonary loading under hypoxic and hypercarbic burrow conditions and support overall stronger Hb-O_2 binding affinity (Hb-O_2 curve is left shifted) for the species tested in this study compared with non-fossorial species.[30]

Our normoxic blood Hb and Hct measurements agree well with previous measurements in these species under normoxic conditions.[30] A novel finding was that Hct increased significantly in hypoxia in all *C hottentotus* species (Table 4). These increases, however, only brought the Hct in these species up to the levels found in normoxia in other species. The elevated levels of Hct and Hb observed in all species in hypoxia (43%-52%) should certainly contribute to their hypoxia tolerance. There were also modest increases in blood lactate concentration in three of the mole-rat species, but this was only significant in *C.h.h.* where levels still did not exceed 10-12 mg dL^{-1}. This suggests that anaerobic metabolism is not a major contributor to the hypoxia tolerance seen in these species until perhaps the very lowest levels of O_2 are reached.

3.5 Study limitations

In this study we compared mole-rats that were found across various regions of Africa, which vary in altitude and habitat conditions, which could in turn influence ventilatory responses to hypoxia. All mole-rats in this study were collected near sea level (~140 m above sea level), with the exception of *C.h.p.* and *H.e.* (collected ~ 1500 m above sea level), but the altitudinal distribution of these mole-rats does not appear to influence their ventilatory responses to hypoxia, as *C.h.p.* exhibited similar ventilatory sensitivity and metabolic responses to hypoxia as the other mole-rats in its species (*C.h.m.*, *C.h.n.*, *C.h.h.*). In addition, the ventilatory and metabolic responses of *H.e.* to hypoxia did not deviate greatly from the other mole-rats in this study, but the blunted breathing frequency response to moderate and severe levels of hypoxia could be a result of the altitude at which this species is found and the O_2 composition of its burrow, which has been recorded to be lower than those of other burrows of mole-rats in this study (Table 1).

The habitats that mole-rats live in vary in many different abiotic factors above ground[31] (Table 1), but the burrow system is thought to provide a relatively thermostable environment.[32] Respiratory gas concentrations are expected to be both more extreme and more variable within burrows than on the surface, but are ultimately thought to depend on factors such as soil type and architectural features of the burrow (ie length, volume, depth, number of entrances, number of occupants, etc).[10, 33, 34] Unfortunately, we do not have information on soil density and architectural features of the burrows of most of the mole-rats used in this study, but based on the general information we have collected from the literature about our mole-rat species[31] (Table 1), there does not appear to be a great influence of these parameters on our findings. Further research investigating how these parameters could influence hypoxia tolerance and ventilatory sensitivity to hypoxia is of great interest.

4 CONCLUSION

The blunted ventilatory response observed in NMRs appears to be an adaptation that is unique to that species of mole-rat. However, the eight closely related mole-rat species

studied here exhibited significant ventilatory responses to hypoxia only in severe hypoxia, suggesting some trait conservation or convergent evolution. Similar ventilatory responses are common in other fossorial species. In addition, the mole-rat species examined here also reduce aerobic metabolic rate and body temperature in severe hypoxia. While this is a trait also shared with most other fossorial and semi-fossorial species, the reductions in aerobic metabolism observed here were greater than those seen in most other fossorial species. Previously, NMRs were identified as the "champion" of hypoxia tolerance, but closely related species of African mole-rats reported in this study, with the exception of *B.s.*, share this physiological trait.

5 MATERIALS AND METHODS

5.1 Animals

Males and females from eight mole-rat species of mole-rat were studied during February of 2018 at the University of Pretoria (~1500 m above sea level): 10 Mahali's mole-rats; *C.h.m.* (103.8 ± 6.2 g), 9 Highveld mole-rats; *C.h.p.* (113.1 ± 9.2 g), 10 Common mole-rats; *C.h.h.* (88.5 ± 5.2 g), 10 Natal mole-rats; *C.h.n.* (117.5 ± 8.8 g), 10 Cape mole-rats; *G.c.* (155.0 ± 17.8 g), 10 Damaraland mole-rats; *F.d.* (125.8 ± 14.8 g), 10 Cape dune mole-rats; *B.s.* (758.9 ± 75.4 g) and 5 Silvery mole-rats *H.e.* (191.2 ± 16.8 g). All mole-rats were held in standard holding conditions (24-25°C, 12h:12h light-dark photoperiod) with unlimited access to food and water. All experimental procedures followed guidelines established by the Canadian Council on Animal Care, and were approved by institutional animal care committees (South Africa care code: ECO69-17; University of Ottawa animal care protocol number 2535). African mole-rat species were wild-caught, and transported to the University of Pretoria, as approved under care code ECO69-17.

5.2 Acute hypoxia responses

We measured the ventilatory and metabolic responses to acute hypoxia using plethysmography and respirometry techniques similar to those used previously for mole-rats and other small mammals.[22, 35-37] Mole-rats were individually placed, unrestrained, inside a 1 L (*C.h.m.*, *C.h.p.*, *C.h.h.*, *F.d.*, *G.c.* and *H.e.*) or 4.7 L (*B.s.*) Plexiglas experimental chamber, held at approximately 28°C, and with a thin layer of bedding on the floor. Mole-rats were given 60-90 minute to adjust to the chamber (when they exhibited a noticeably relaxed and stable breathing pattern) before measurements began. Ambient air (~18 kPa O_2, the partial pressure of O_2 at ~ 1500 m above sea level) was supplied to the animal chamber at a flow rate of 600 mL min^{-1} (small chamber) or 2 L min^{-1} (large chamber). Measurements of breathing and metabolism in these ambient conditions were then recorded for an additional 30 minute, after which mole-rats were exposed to 30 minute stepwise decreases in inspired O_2 tension (PO_2): 12, 9, 7, 5, 3 and 2 kPa. *B.s.* was only tested to 5 kPa O_2 as they exhibited signs of discomfort and stress at 3 kPa, whereas *H.e.* was the only species tested to 2 kPa O_2. Dry incurrent air and nitrogen were mixed using pre-calibrated rotameters (Matheson Model 7400 Gas Mixer, E700 and E500 flowtubes) and calibrated mass flow meters (Alicat Scientific) to achieve each level of hypoxia. Body temperature was measured non-invasively every 10 minute from previously implanted subcutaneous RFID microchips using RFID readers (Destron Fearing).

Metabolism, breathing and body temperature were measured continuously throughout each experiment, and we analysed and report the average values across the last 10 minute at each inspired PO_2 level. The excurrent air leaving the animal chamber was subsampled at 200 mL min^{-1}, analysed for water vapour (RH-300; Sable Systems), dried with pre-baked drierite (Drierite, WA Hammond Drierite Co. Ltd.) and analysed for CO_2 and O_2 fraction using an infrared CO_2 analyser and a galvanic fuel cell O_2 analyser (FOXBOX, Sable Systems). These data were used to calculate rates of O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$), expressed at standard temperature and pressure (STP), as recommended by Lighton.[38] Chamber temperature was continuously recorded with a thermocouple (PT-6; Physitemp). Breathing frequency and tidal volume were measured from changes in pressure between the animal chamber and reference chamber (which arise from the warming and humidifying of the air as it is inspired by the animal), and was detected using a differential pressure transducer (Validyne DP45; Cancoppas) connected between the two chambers. Breathing frequency was calculated directly from the ventilation-induced pressure oscillations. Tidal volume was calculated using established equations and expressed as STP.[39, 40] Total ventilation was determined as the product of breathing frequency and tidal volume, ventilatory equivalent for O_2 or CO_2 was calculated by dividing total ventilation by $\dot{V}O_2$ or $\dot{V}CO_2$, respectively, and pulmonary O_2 extraction (%) was calculated as $\dot{V}O_2$ divided by the product of total ventilation and inspired PO_2. All data were acquired using a PowerLab 16/32 and LabChart 8 Pro software (ADInstruments).

5.3 Blood sampling

Mole-rats were sampled for blood measurements (not all species were sampled, and for some species only a subset): 10 *C.h.m.*, 10 *C.h.p.*, 10 *C.h.h.*, 10 *G.c.*, 3 *H.e.* and 7 *B.s.* Before sampling, animals were exposed to either (a) 3 hours of normoxia (18 kPa O_2; control) or (b) 3 hours of hypoxia (5 kPa O_2 for *C.h.m.*, *C.h.p.*, *C.h.h.* and *G.c.* or 7 kPa O_2 for *B.s.*; following a 30 minute step to 12 kPa O_2). Hypoxia levels were chosen as being just above the maximum hypoxia tolerance of the animals, as previously determined (above). Animals were monitored throughout using the same equipment as above. Following these exposures, animals were rapidly killed by cervical dislocation followed by decapitation; and blood pH, HCO_3^- (mmol L^{-1}) and lactate (mg dL^{-1}) were analysed ex vivo using $CG4^+$ cartridges with the iStat VetScan Analyzer (Abaxis). Blood was also collected in heparinized capillary tubes and spun for Hct or collected for Hb measured using Drabkin's reagent (Sigma-Aldrich), according to the manufacturer's instructions. Given the method of collection, the blood collected was a mix of arterial and venous blood.

5.4 Lung volume and histology

Lung volume was measured and histology conducted on the mole-rats sampled above. Lungs were inflated with PE50 tubing inserted and secured to the trachea, and 10% formalin infused at a pressure of 30 cmH_2O.[41] Once lungs stopped growing in size, the trachea was tied off and lungs were removed from the body, with volume measured using the fluid displacement technique.[41, 42] Lungs remained in 10% formalin for 3 days before being transferred into 75% ethanol and then wax embedded. Samples were sectioned at the middle region of the left lung lobe at 5 µm and H&E stained. Images were acquired using light microscopy at 20 × magnification and collected systematically such that there was

equal representation of images from across the lobe and sufficient images were analysed for each sample to account for heterogeneity (determined by the number of replicates necessary to yield a stable mean linear intercept).[42, 43] Total alveolar surface area was determined from previously described equations,[44] using mean alveolar density, calculated from mean linear intercept,[45, 46] and lung volume.

5.5 Statistics

One-factor repeated measure ANOVAs were used to examine the main effects of acute inspired PO_2 within each mole-rat species. Holm-Sidak post-tests were used, when appropriate, to assess changes in variables from normoxic/resting conditions. Lung volume and morphometrics were analysed using one-factor ANOVAs to compare between species. Blood parameters were analysed using t tests to compare normoxia vs hypoxia exposed individuals within a species, to provide insight into how each species responds to hypoxia challenge. Values are reported as mean ± SEM. All statistical analysis was conducted with SigmaStat software (v. 3.5) with a significance level of $P < .05$. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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