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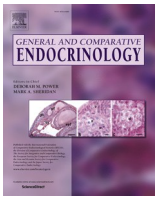

Short communication

The relationship between hypoxia exposure and circulating cortisol levels in social and solitary African mole-rats: An initial report

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ABSTRACT

Hypoxemia from exposure to intermittent and/or acute environmental hypoxia (lower oxygen concentration) is a severe stressor for many animal species. The response to hypoxia of the hypothalamic–pituitary-adrenal axis (HPA-axis), which culminates in the release of glucocorticoids, has been well-studied in hypoxia-intolerant surface-dwelling mammals. Several group-living (social) subterranean species, including most African mole-rats, are hypoxia-tolerant, likely due to regular exposure to intermittent hypoxia in their underground burrows. Conversely, solitary mole-rat species, lack many adaptive mechanisms, making them less hypoxia-tolerant than the social genera. To date, the release of glucocorticoids in response to hypoxia has not been measured in hypoxia-tolerant mammalian species. Consequently, this study exposed three social African mole-rat species and two solitary mole-rat species to normoxia, or acute hypoxia and then measured their respective plasma glucocorticoid (cortisol) concentrations. Social mole-rats had lower plasma cortisol concentrations under normoxia than the solitary genera. Furthermore, individuals of all three of the social mole-rat species exhibited significantly increased plasma cortisol concentrations after hypoxia, similar to those of hypoxia-intolerant surface-dwelling species. By contrast, individuals of the two solitary species had a reduced plasma cortisol response to acute hypoxia, possibly due to increased plasma cortisol under normoxia. If placed in perspective with other closely related surface-dwelling species, the regular exposure of the social African mole-rats to hypoxia may have reduced the basal levels of the components for the adaptive mechanisms associated with hypoxia exposure, including circulating cortisol levels. Similarly, the influence of body mass on plasma cortisol levels cannot be ignored. This study demonstrates that both hypoxia-tolerant rodents and hypoxia-intolerant terrestrial laboratory-bred rodents may possess similar HPA-axis responses from exposure to hypoxia. Further research is required to confirm the results from this pilot study and to further confirm how the cortisol concentrations may influence responses to hypoxia in African mole-rats.

1. Introduction

During stressful conditions, several mechanisms are activated as part of homeostatic control, including behavioural, visceral and endocrine changes (Arias-Reyes et al., 2021; Zoccal et al., 2007). For example, in mammals, the primary endocrine response to stress is the activation of the hypothalamic–pituitary-adrenal axis (HPA-axis), which culminates in the release of glucocorticoids (cortisol or corticosterone) from the adrenal glands into the circulation throughout the body (Herman et al., 2016). One such stressor is exposure to physiologically demanding atmospheric conditions, such as intermittent and acute hypoxia (lower oxygen concentrations). Typically, surface-dwelling mammals, such as

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terrestrial laboratory-bred rodents (e.g. *Mus musculus* and *Rattus norvegicus*), belonging to the rodent suborder Myomorpha, rarely experience hypoxia, likely only during a temporary cessation of respiratory airflow (i.e., Apnea) or due to various pathologies (e.g., COPD) (Bodager et al., 2014). In comparison, species confined to underground burrows (i.e. subterranean mammals) are presumably regularly exposed to challenging atmospheric conditions. Indeed, in the subterranean burrows of social species, where numerous animals live within the confined space of the nest that is often at depth, and there is poor gas diffusion through the surrounding soils, the atmosphere may become intermittently or chronically hypoxic and/or hypercapnic (Roper et al., 2001). As a result of this exposure, subterranean mammalian species, and particularly those that live in groups, may have evolved to be more tolerant to hypoxia (so-called hypoxia-tolerant species) than surface-dwelling mammals (so-called hypoxia-intolerant species) (Frappell et al., 1992).

Activation of the HPA-axis and the subsequent release of glucocorticoids in response to intermittent and/or acute hypoxia is well documented in hypoxia-intolerant species, with the most common shared response being an increase of up to 200% in circulating glucocorticoids (Bodager et al., 2014; Bruder et al., 2011, 2008; Chintamaneni et al., 2013; Guenther et al., 2012; Hwang et al., 2017; Johnson et al., 2013; Zoccal et al., 2007). Yet, surprisingly, the HPA-axis response of hypoxia-tolerant species to intermittent and/or acute hypoxia has never been investigated.

African mole-rats, belonging to the rodent suborder Hystricomorpha, provide a unique rodent model system as they exhibit different degrees of sociality, ranging from solitary to eusocial, as well as a range of hypoxia tolerances (Bennett and Faulkes, 2000, Ivy et al. 2020). All African mole-rat species are exclusively subterranean and thus share many similar environmental conditions in their burrows, although these conditions vary with group size (Bennett et al., 1988; Bennett and Faulkes, 2000; Holtze et al., 2018). As a result, mole-rats, and particularly the naked mole-rat (*Heterocephalus glaber*), which occurs in large groups of approximately 80 individuals, exhibit robust adaptations to anoxia and hypoxia (Ilacqua et al., 2017; Pamenter et al., 2015; Pamenter, 2022; Park et al., 2017). Similarly, several recent studies have suggested that other social mole-rat species, such as those of the genus *Cryptomys* (that live in groups of up to 20 individuals (Hart et al. 2021)), also have remarkable adaptations to hypoxia, resulting in elevated hypoxia tolerance relative to the mole-rat species that live a solitary lifestyle (Ivy et al., 2020; Logan et al., 2020). Importantly, solitary mole-rat species only pair up briefly during the mating season to court and breed, and the offspring subsequently disperse from the natal burrow after weaning and are expected not to be exposed to frequent bouts of hypoxia thereafter (Bennett and Jarvis, 1988; Hart et al., 2006). However, these solitary species would still experience more frequent hypoxia exposure than exclusively terrestrial species.

In the present study, we aimed to address the dearth of knowledge pertaining to the response of the HPA-axis of hypoxia-tolerant subterranean mammals to exposure to acute hypoxia. To this end, we exposed three social mole-rat species, the naked mole-rat, the common mole-rat (*Cryptomys hottentotus hottentotus*) and the highveld mole-rat (*C. h. pretoriae*), as well as two solitary mole-rat species, the Cape dune mole-rat, (*Bathyergus suillus*), and the Cape mole-rat, (*Georychus capensis*), to normoxia or acute hypoxia and measured their circulating plasma cortisol (the primary glucocorticoid in African mole-rats (Bennett and Faulkes, 2000)) concentrations.

2. Materials and methods

(a) Study animals.

Apart from the naked mole-rats, which came from laboratory-raised colonies, all the other mole-rat species were wild-caught in South Africa. All study animals were adults, and only non-breeding colony members were used for the social mole-rat species. Work with solitary species was performed outside their breeding season. Wild-caught animals were in captivity for seven to ten days prior to initiating the experimental protocol. All other details on the methods employed are provided in the electronic supplementary materials.

(b) Experimental protocol.

Animals were randomly assigned to either *i*) 3 h of normoxia at 18.5 kPa O_2 (wild-caught mole-rats in Pretoria) or 21 kPa O_2 (captive-bred naked mole-rats in Ottawa) (balance N_2; control), or *ii*) 3 h of hypoxia at 5 kPa (5% O_2) for most species or 7 kPa (7% O_2) for Cape dune mole-rats. Hypoxia exposures were preceded by a 30 min step at 12 kPa O_2 and were chosen to be just above the minimum tolerable O_2 threshold that the animals could withstand in preliminary experiments (Ivy et al., 2020). Following experimentation, animals were immediately euthanised by cervical dislocation followed by decapitation (See supplementary material for additional details). Whole blood was collected directly from the neck and/or heart using a 3.0 ml syringe with a 25-gauge × 5/8-inch needle. After collection, the blood was transferred into a Vacutainer coated with lithium heparin. The blood collection tubes were subsequently centrifuged at 3000 rpm for 15 min. Plasma was collected and stored at − 20 °C for cortisol analysis.

(c) Plasma cortisol analysis.

Plasma cortisol concentrations were determined using a commercially available coated tube assay kit. The assay was carried out according to the manufacturer's protocol. Further details are provided in the electronic supplementary material.

(a) Statistical analysis.

Each of the five species was analysed separately, owing to the significant differences between species in the plasma cortisol titres of the mole-rats exposed to normoxia (Kruskal-Wallis: $H = 17.6$, $df = 4$, $p = 0.002$, Fig. 1). Normality of the plasma cortisol concentrations for each species was determined using Shapiro-Wilk tests, and homogeneity of normally distributed dependent variables was confirmed with a Levene's test. Log-transformation was attempted to normalise all non-normally distributed dependent variables. On log transformation, all data met the normality assumption; consequently, an independent *t*-test was conducted to compare plasma cortisol concentrations of animals exposed to normoxia or acute hypoxia for each species. To confirm the above analysis, a generalised linear model, with gamma distributions and link-identity function from the *lme4* package, was used to analyse differences between the five species and treatments (normoxia, hypoxia) and the two-way interaction species and treatment. *Post-hoc* comparisons of significant interactions were obtained by Tukey's HSD (honestly significant difference) test (See supplementary material for results). All statistical analyses were performed in R 3.5.2 (R Development Core Team, 2018). The results herein are presented as mean ± standard error.

3. Results

The solitary species (106.3 ± 24.9 ng/ml) were observed to possess 666% higher plasma cortisol concentrations under normoxia than the social species (16.0 ± 3.05 ng/ml) (Fig. 1, See supplementary material for results). All social mole-rats species exhibited a significant increase in plasma cortisol concentrations following acute *in vivo* hypoxia exposure relative to normoxic control animals (Table 1, Fig. 1). Plasma cortisol increased by 428 ± 115% in social species exposed to hypoxia (naked mole-rat – 266%; common mole-rat – 368%; highveld mole-rat – 650%, Fig. 1). By contrast, both solitary species had statistically similar levels of cortisol concentrations between hypoxia and normoxia (Table 1, Fig. 1). Solitary species exhibited an upward trend of plasma cortisol under hypoxia (158 ± 16% increase; Cape dune mole-rat – 142%; Cape mole-rat – 172%), but this increase was much lower than the social species.

4. Discussion

The study was undertaken to unravel the relationship between hypoxia exposure and circulating cortisol levels in social and solitary

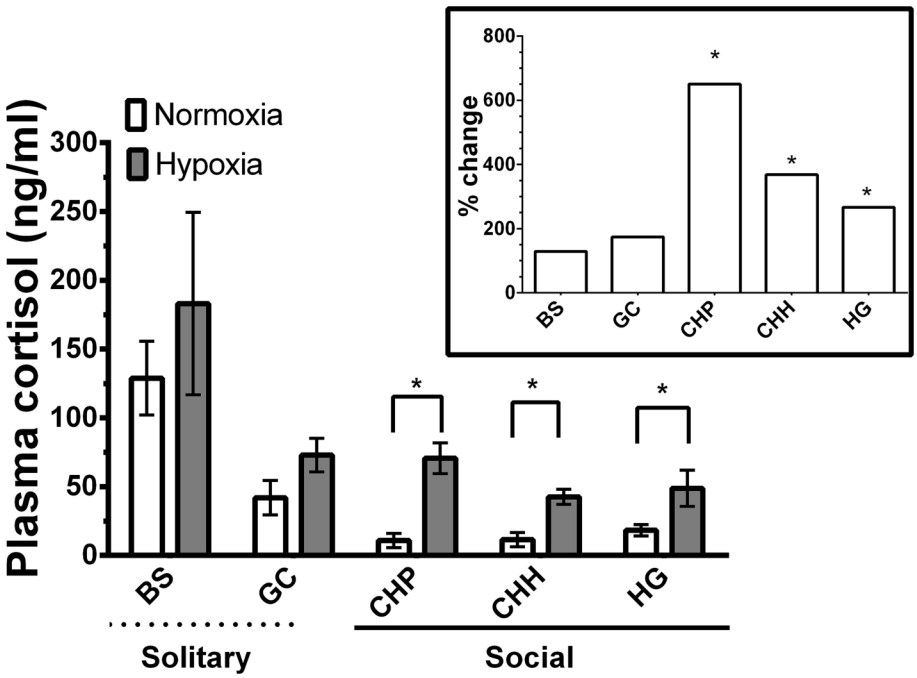

Fig. 1. The plasma cortisol concentration (ng/ml) of five African mole-rat species (three social and two solitary species) exposed to normoxia or acute hypoxia. Insert: Percentage increase (% change) in plasma cortisol concentration of mole-rats exposed to acute hypoxia compared to those exposed to normoxia. BS – Cape dune mole-rat; GC – Cape mole-rat; HG – naked mole-rat; CHH – common mole-rat; CHP – highveld mole-rat. The results are presented as mean ± standard error. Asterisks (*) indicate a significant difference ($p \leq 0.05$).

Table 1
Summary of animal numbers used and statistical results of *t*-test results comparing plasma cortisol concentrations in animals exposed to normoxia or acute hypoxia.

Species	Sociality	n		t	p
		Normoxia	Hypoxia		
Naked mole-rat	Social	18	12	3.11	0.005*
Common mole-rat	Social	5	4	6.73	0.005*
highveld mole-rat	Social	4	7	8.12	0.001*
Cape mole-rat	Solitary	5	3	5.24	0.13
Cape dune mole-rat	Solitary	6	3	0.76	0.51

(*) indicates significant difference from normoxia ($p \leq 0.05$).

African mole-rats. Firstly, the findings imply that species of Hystricomorpha with smaller body sizes which are historically exposed to a high frequency of intermittent hypoxia, the social African mole-rat species, may reduce the basal levels of the components of the adaptive mechanisms associated with hypoxia exposure, including circulating cortisol levels. Secondly, and likely tightly linked to the finding above, this study indicates that both hypoxia-tolerant rodents, including one of the most hypoxia-tolerant mammals (the naked mole-rat), and hypoxia-intolerant terrestrial laboratory-bred rodents, may possess a similar HPA-axis response from exposure to hypoxia.

All social mole-rat species exhibit a significant increase in plasma cortisol levels when exposed to acute hypoxia, whereas the solitary species do not. Specifically, cortisol increases > 200% in social mole-rats, which is similar to previous observations from similarly-treated hypoxia-intolerant rodents (Hwang et al., 2017; Zoccal et al., 2007), whereas cortisol increases < 200% in the solitary species.

However, the reduced plasma cortisol response to acute hypoxia in solitary African mole-rat species may be due to their increased normoxia plasma cortisol titres compared to social species that occur in organised families. This study is one of the first to investigate circulating cortisol levels in solitary mole-rats, and therefore it was surprising to observe this significant difference between circulating cortisol levels of solitary and social mole-rats species under normoxia (666% difference). Unfortunately, this study did not set out to conclusively investigate the possible causes of this difference; however, we have attempted to set out possible causes that future studies could use as a starting point to investigate this unique pattern.

Firstly, a link between plasma cortisol levels and metabolic rate in mammals has been highlighted; namely, baseline plasma cortisol concentrations vary with the mass-specific metabolic rate among cortisol-dominant mammals (Haase et al., 2016). Haase et al. (2016) suggested a linear relationship between plasma cortisol concentrations and mass-specific metabolic rate, with mammal species possessing a higher mass-specific metabolic rate possessing higher plasma cortisol concentrations. African mole-rats are renowned for possessing lower mass-specific resting metabolic rates than terrestrial-dwelling rodents (Bennett and Faulkes, 2000; Ivy et al. 2020; Wallace et al. 2021; Hart et al. 2022a). As with all mammal species, the heavier species of African mole-rats, namely the solitary species, possess a lower mass-specific metabolic rate than the lighter species of African mole-rats, namely the social species (Šumbera 2019; Ivy et al. 2020; Hart et al. 2022a). However, in contrast to Haase et al. (2016) suggestion, the solitary mole-rat species, which possess the lowest mass-specific metabolic rate, possessed the highest circulating cortisol concentrations. Secondly, Haase et al. (2016) also alluded to the link between circulating cortisol concentrations and body mass between mammalian species. A positive relationship between higher body masses and higher plasma cortisol titre was observed in cortisol-dominant mammals (Haase et al., 2016). This trend was observed within this study on African mole-rats (see Figure S1); however, as this study only includes five African species, with one species, the Cape Dune mole-rat, vastly outweighing the other mole-rat species of this study, we hesitate to speculate more on this pattern, however further research is warranted to investigate such a trend. Thirdly, repeated exposure to a stressful event (a chronic stressor), such as intermittent hypoxia, may result in extended periods of high levels of circulating glucocorticoids, ultimately resulting in detrimental long-term effects, such as reduced immune system strength and reproductive capacity (Bauer, 2005; Hart et al., 2022b). This would be incompatible with these species thriving in such a natural environment, and as such, a reduction in basal cortisol circulation may have been selected to counter the possibility of chronically high cortisol levels due to exposure to intermittent hypoxia in social African mole-rat species. As such, the solitary African mole-rat species, still being exposed to hypoxia but less frequently, would possess higher basal cortisol than social species as they are at a lower risk of HPA-exhaustion.

Interestingly, similar patterns between social and solitary African mole-rat species have been observed in other biological processes relating to hypoxia exposure, including thermogenesis and erythropoiesis (Cheng et al., 2021; Ivy et al., 2020). For example, social African mole-rat species rapidly down-regulate energetically-demanding non-shivering thermogenesis through reduced expression of uncoupling protein-1 (UCP1)-mediated mitochondrial uncoupling in brown adipose tissue (BAT), while upregulating erythropoiesis resulting in increased haematocrit and haemoglobin during acute *in vivo* hypoxia exposure to increase oxygen transport (Cheng et al., 2021; Ivy et al., 2020). Conversely, the solitary Cape mole-rat does not down-regulate UCP1 in BAT in acute hypoxia (Cheng et al., 2021), while both the Cape dune and Cape mole-rats do not exhibit changes in haematocrit and haemoglobin when exposed to hypoxia (Ivy et al., 2020). Again this may be due to increased UCP1 expression, haematocrit, and haemoglobin of solitary African mole-rat species under normoxia. As with the plasma cortisol results of the current study, basal (under normoxia) levels of UCP1 expression, haematocrit, and haemoglobin of solitary African mole-rat species are higher than those of the social species (Cheng et al., 2021; Ivy et al., 2020). This, again, may indicate that selective pressure is placed on social African mole-rat species by frequent exposure to intermittent hypoxia, which may reduce the basal levels of signalling intermediates underlying adaptive mechanisms associated with hypoxia exposure.

Unfortunately, testing this hypothesis is difficult because there are no exclusively aboveground African mole-rats species in which, according to the hypothesis above, we would predict raised cortisol, UCP1, haematocrit and haemoglobin levels under normoxia. However, a well-studied, social, primarily surface-dwelling (non-fossorial) member of the suborder Hystricomorpha may be essential to unlocking this hypothesis. Guinea pigs (*Cavia aperea*), in the suborder Hystricomorpha, are often kept as pets while also being a useful model for biomedical research and, like African mole-rats, utilise cortisol as their primary glucocorticoid (Rystrom et al., 2022). Interestingly, in basal normoxic environments, guinea pigs express higher circulating plasma cortisol concentrations (Mean: ~690 ng/ml; range: 134–1440 ng/ml) (Rystrom et al., 2022) relative to those of all African mole-rats species of this study (Fig. 1). Therefore, a pattern may be apparent whereby the normoxia basal circulating cortisol concentration is low in Hystricomorpha species exposed frequently to intermittent hypoxia, possibly to avoid HPA-exhaustion. Unfortunately, there has been no investigation into the regulation of the HPA-axis, and ultimately cortisol response, or UCP1 expression in response to hypoxia in adult guinea pigs, but changes in haematocrit and haemoglobin values in response to acute hypoxia have been recorded (Docio et al., 2018; Ederstrom et al., 1971; Genzer et al., 2019; Lechner et al., 1981; Spittler et al., 2021). Interestingly, the normoxic baseline and hypoxia-treated haematocrit and haemoglobin values of adult guinea pigs are similar to those of solitary African mole-rats (Docio et al., 2018; Ederstrom et al., 1971; Genzer et al., 2019; Ivy et al., 2020; Lechner et al., 1981; Spittler et al., 2021). This again implies that species of Hystricomorpha which are historically exposed to a high frequency of intermittent hypoxia, the social African mole-rat species, may reduce the basal levels of the components of the adaptive mechanisms associated with frequent hypoxia exposure. Guinea pigs have been suggested to have a higher tolerance to hypoxic environments than laboratory-bred rodents (Gonzalez-Obeso et al., 2017), but this has been contested (Lechner et al., 1981); however, they have never been directly compared to African mole-rats. However, it must be highlighted that the guinea pigs apart of Rystrom et al. (2022) study were heavier than the mole-rats species of this study (Figure S2), and as such, we can not disqualify the effect of body mass on plasma cortisol levels under normoxia. Further work on guinea pigs and the suborder Hystricomorpha under hypoxia may provide further insight into this question.

However, this study's findings and conclusions must be considered through a pilot study's scope. Therefore, a greater sample size for all African mole-rat species is necessary to test these preliminary findings robustly. Furthermore, since only plasma cortisol levels were measured, we cannot speculate on regulation by receptor expression and/ or affinity as well as 'crosstalk' between different biological processes; therefore, it is not possible to conclude how the cortisol concentrations may influence responses to hypoxia in African mole-rat. Furthermore, from a phylogenetic point of view, similar research on more closely related species across a range of body masses, such as those belonging to the suborder Hystricomorpha, including guinea pigs, dassie rats (*Petromus typicus*), and the cane rat (*Thyronomys*) (Smith et al., 2015), are needed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Ethical statement

All experimental procedures conducted in Canada were approved by the University of Ottawa Animal Care Committee (protocol #2535), in accordance with the Animals for Research Act and by the Canadian Council on Animal Care. Trapping and experiments done in South Africa were conducted under appropriate permits issued by Cape Nature Conservation and the Department of Nature Conservation in the Western Cape, Republic of South Africa (CN44-31–2285) and with experimental procedures approved by the animal ethics committee of the University of Pretoria (EC069-17).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2023.114294.

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