Evolved changes in breathing and CO_2 sensitivity in deer mice native to high altitudes

Catherine M. Ivy* and Graham R. Scott

Department of Biology, McMaster University, Hamilton, ON, Canada

* Corresponding author:

Catherine M. Ivy

ivycm@mcmaster.ca

Running Head: Control of breathing in high-altitude deer mice

Key words: hypercapnic ventilatory response, hypoxia acclimation, hypoxic ventilatory response, generational effects

Summary Statement (15-30 words): Evolved changes in breathing and O_2 uptake in high-altitude deer mice are not associated with any changes in ventilatory CO_2 sensitivity.

ABSTRACT

We examined the control of breathing by O_2 and CO_2 in deer mice native to high altitude, to help uncover the physiological specializations used to cope with hypoxia in high-altitude environments. Highland deer mice (*Peromyscus maniculatus*) and lowland white-footed mice (*P. leucopus*) were bred in captivity at sea level. The first- and second-generation progeny of each population were raised to adulthood and then acclimated to normoxia or hypobaric hypoxia (12 kPa O_2, simulating hypoxia at ~4300 m) for 6-8 weeks. Ventilatory responses to poikilocapnic hypoxia (stepwise reductions in inspired O_2) and hypercapnia (stepwise increases in inspired CO_2) were then compared between groups. Both generations of lowlanders appeared to exhibited ventilatory acclimatization to hypoxia (VAH), in which hypoxia acclimation enhanced the hypoxic ventilatory response and/or made breathing pattern more effective (higher tidal volumes and lower breathing frequencies at a given total ventilation). In contrast, hypoxia acclimation had no effect on breathing in either generation of highlanders, and breathing was generally similar to hypoxia-acclimated lowlanders. Therefore, attenuation of VAH appears to be an evolved feature of highlanders that persists for multiple generations in captivity. Hypoxia acclimation increased CO_2 sensitivity of breathing, but in this case the effect of hypoxia acclimation was similar in highlanders and lowlanders. Our results suggest that highland deer mice have evolved high rates of alveolar ventilation that are unaltered by exposure to chronic hypoxia, but they have preserved ventilatory sensitivity to CO_2.

INTRODUCTION

Animals native to high altitude can provide insight into the evolution of complex physiological systems, because they have often adapted to the stressors associated with this challenging environment. High altitude is both cold and hypoxic, which challenges the ability of endotherms to maintain O_2 supply for thermoregulation and exercise. However, many human, other mammal, and bird populations live, reproduce, and exercise at high altitude, and the emerging evidence suggests that they have overcome the challenges of this environment through evolved changes in the O_2 transport cascade (30, 54). The function of this cascade – composed of ventilation, pulmonary diffusion, circulation, tissue diffusion, and cellular O_2 utilization – relies on adequate rates of ventilation to maintain tissue O_2 supply; therefore, increasing breathing is critical for O_2 uptake in hypoxic environments (54).

Breathing is stimulated by reductions in arterial O_2 levels at high altitude. Ventilation increases in response to acute hypoxia challenge, a process termed the hypoxic ventilatory response (HVR). Peripheral chemoreceptors in the carotid bodies sense reductions in the partial pressure of O_2 (PO_2) in arterial blood, which initiates the hypoxic chemoreflex that results in the HVR (10, 41). Breathing and ventilatory O_2 chemosensitivity is further enhanced with prolonged exposure to hypoxia over days to weeks, a process termed ventilatory acclimatization to hypoxia, (VAH). VAH is believed to result from increases in chemosensitivity of the carotid bodies, and from increases in central gain of the afferent signals transmitted from the carotid bodies to the brainstem (35, 36, 43, 57). The resulting increases in ventilation improve O_2 uptake by increasing alveolar and arterial PO_2 (15).

Breathing at high altitude is also modulated by arterial CO_2 levels. The increases in ventilation that act to minimize the fall in arterial PO_2 also lead to a decline in the partial

pressure of CO_2 (PCO_2) (respiratory hypocapnia) (39, 47). This can reduce the CO_2 chemoreflex drive to breathe, acting as feedback that inhibits the ventilatory response to environmental hypoxia (39). As a result, the HVR measured in poikilocapnic (uncontrolled CO_2) conditions is generally lesser in magnitude than when the HVR is measured under isocapnic conditions (when arterial PCO_2 is experimentally maintained) (32, 48). Furthermore, exposure to chronic hypoxia can increase ventilatory CO_2 sensitivity (7, 8, 27, 44, 52). Therefore, CO_2 sensitivity can have a strong influence on breathing and O_2 uptake in individuals at high altitude.

How has the control of breathing been adjusted in high-altitude natives? The isocapnic HVR as a measure of O_2 chemosensitivity has been examined in some studies of highland human populations, but the HVR of most other highland species has been examined in poikilocapnic conditions. Nevertheless, the literature suggests that highland natives can differ from lowland natives in divergent ways, with some highlanders exhibiting similar or enhanced ventilatory responses (22, 37, 48) and others exhibiting a blunted HVR (18, 21, 45). However, in these studies it has often been difficult to distinguish uniquely evolved differences in highland taxa from developmental or multigenerational effects of exposure to hypoxia (1, 31). Much less is known about CO_2 sensitivity of breathing in highland taxa. Highland humans appear to have a reduced ventilatory sensitivity to CO_2 (49, 50), but it is unclear whether other high-altitude taxa exhibit a similar or distinct pattern of CO_2 sensitivity.

The objective of this study was to examine how the control of breathing by O_2 and CO_2 has evolved in high-altitude populations of deer mice (*Peromyscus maniculatus*). Deer mice are broadly distributed across North America and can be found from sea level to over 4,300 m elevation in the Rocky Mountains (14, 33, 51). High-altitude populations must sustain high metabolic rates in the wild (11), and they have evolved a higher aerobic capacity ($\dot{V}O_2max$) in

hypoxia than their low-altitude counterparts (2, 3, 25, 55) in association with changes in haemoglobin-O_2 affinity, cardiac function, muscle capillarity and metabolic phenotype, and tissue gene expression (4, 25, 26, 34, 46, 51, 53, 55, 56). We recently found that the control of breathing also differs in high-altitude deer mice compared to a congeneric species from low altitude (white-footed mouse, *P. leucopus*), in a study of animals that were born and raised in captivity at sea level but were the first-generation progeny of wild parents (16). Specifically, we found that highlanders do not appear to exhibit VAH, in contrast to the robust VAH exhibited by lowlanders, but that highlanders have a fixed breathing pattern that is similar to hypoxia-acclimated lowlanders (16). However, because these observations were made in first-generation progeny, it was unclear whether they resulted from an evolved difference in highlanders or from persistent multigenerational effects of the wild parents being born and raised in different native environments. Here, we sought to examine these possibilities by studying mice from both the first and second generations raised in captivity. We also sought to determine whether variation in CO_2 sensitivity has evolved in high-altitude mice, and whether this might contribute to the apparent differences in breathing during poikilocapnic hypoxia and in VAH.

MATERIALS AND METHODS

Mouse populations. Wild adult mice were live trapped at low altitude on the Great Plains (Nine Mile Prairie, Lancaster County, NE, at 40°52’12’’N, 96”48’20.3’’W, 430 m above sea level) (*P. leucopus*) and at high altitude on the summit of Mount Evans (Clear Creek County, CO, at 39°35’18’’N, 105°38’38’’W, 4,350 m above sea level) (*P. maniculatus rufinus*), and were then transported to McMaster University (Hamilton, ON, Canada; ~50 m above sea level) and held in captivity. Mice were bred within each population in common conditions to produce first-

generation (G1) progeny. G1 mice were also bred within each population to produce second-generation (G2) progeny. All captive-born progeny were held in standard holding conditions (24-25°C, 12:12 light-dark photoperiod) with unlimited access to food and water, and were raised in ambient conditions (sea level normoxia) until 6 months of age before experiments were conducted. All animal protocols followed guidelines established by the Canadian Council on Animal Care and were approved by the McMaster University Animal Research Ethics Board.

Acclimation groups. To assess the influence of native population and acclimation environment, mice were chronically exposed to i) standard normobaric normoxia or ii) hypobaric hypoxia simulating the pressure at an elevation of ~4,300 m (barometric pressure of 60 kPa, PO_2 ~12.5 kPa). Specially designed hypobaric chambers were used for exposure to chronic hypoxia, as previously described (16, 28). Mice in hypobaric hypoxia were temporarily returned to normobaric conditions twice per week for <20 min for cage cleaning. Ventilatory measurements were carried out after 6-8 weeks of exposure.

Acute hypoxia responses. We examined the effects of hypoxia acclimation on the response to acute hypoxia in both G1 and G2 mice from each population. Breathing and O_2 consumption rates ($\dot{V}O_2$) were measured in unrestrained mice using barometric plethysmography and respirometry techniques that are consistent with our previous studies (16, 17). Mice were placed in a whole-body plethysmograph with normoxic air (21 kPa O_2, balance N_2) supplied at a rate of 600 ml min^{-1} and were given 20-60 min to adjust to the chamber until relaxed and stable breathing and metabolism was observed. Measurements were then recorded for an additional 20 min at 21 kPa O_2, after which mice were exposed to stepwise reductions in inspired partial

pressure of O_2 (PO_2) at 16, 12, 10, 9, and 8 kPa for 20 min at each step. Incurrent gas composition was set by mixing dry compressed gases using precision flow meters (Sierra Instruments, Monterey, CA, USA) and a mass flow controller (MFC-4, Sable Systems, Las Vegas, NV, USA), such that the desired PO_2 was delivered to the chamber at a constant rate of 600 ml min^{-1}. Body temperature (T_b) was measured at the end of the experiment using a mouse rectal probe (RET-3-ISO, Physitemp). T_b was also measured exactly 24 h later to determine normoxic T_b (this was used as a proxy for the normoxic T_b at the start of the experiment, which was not measured to prevent stress to the animal).

Breathing and $\dot{V}O_2$ were determined during the last 10 min at each PO_2. Incurrent and excurrent air flows were subsampled at 200 ml min^{-1}. For incurrent air, O_2 fraction was continuously measured using a galvanic fuel cell O_2 analyzer (FC-10, Sable Systems). For excurrent air, we first measured water vapour using a thin-film capacitive water vapour analyzer (RH-300, Sable Systems), then dried the gas stream with pre-baked drierite and measured O_2 fraction as above and CO_2 fraction using an infrared CO_2 analyzer (CA-10, Sable Systems). These data were used to calculate $\dot{V}O_2$, expressed at standard temperature and pressure (STP), using appropriate equations for dry air as described by Lighton (24). Chamber temperature was continuously recorded with a thermocouple (TC-2000, Sable Systems). Breathing frequency and tidal volume were measured from changes in flow across a pneumotachograph in the plethysmograph wall, detected using a differential pressure transducer (Validyne DP45, Cancopass, Mississauga, ON, Canada). Tidal volume was calculated using established equations (5, 19) assuming a constant rate of decline in T_b with declining PO_2, which we have previously shown results in similar tidal volumes to those calculated using direct T_b measurements at each PO_2 (17). Total ventilation was determined as the product of breathing frequency and tidal

159 volume. Total ventilation and tidal volume data are expressed at STP. All data was acquired
160 using a PowerLab 16/32 and Labchart 8 Pro software (ADInstruments, Colorado Springs, CO,
161 USA).
162 Mice were returned to their acclimation environment after completing the above protocol
163 and allowed at least 2 days to recover and were then subjected to one of two protocols, to
164 measure either the acute hypoxia response in the presence of elevated inspired CO_2 or acute
165 hypercapnia responses, as described below.
166
167 *Acute hypoxia responses with elevated inspired CO_2.* We measured responses to acute
168 hypoxia in the presence of modestly elevated levels of inspired CO_2 in G1 mice, in order to
169 examine the HVR under conditions in which respiratory hypocapnia was reduced. We used the
170 same whole-body plethysmograph and the same stepwise hypoxia conditions as was used for the
171 acute hypoxia responses that are described above, except that mice were also exposed to a
172 constant incurrent partial pressure of CO_2 (PCO_2) of 2 kPa across all acute hypoxia steps.
173 Breathing and metabolism were determined as described above, except that CO_2 fraction was
174 measured in dry incurrent air for a few minutes at the beginning of each step (to assure a constant
175 incurrent CO_2 baseline at the desired level), after which it was measured in dry excurrent air for
176 the remaining time at each step.
177
178 *Acute hypercapnia responses.* We measured responses to acute stepwise hypercapnia in
179 G2 mice in order to assess ventilatory CO_2 sensitivity. We used the same whole-body
180 plethysmograph and the same conditions as was used for the measurements of acute hypoxia
181 response that are described above, except that breathing and metabolism were measured during

acute step-wise increases in environmental PCO_2 at 0, 2, 4, and 6 kPa CO_2. These measurements were made once in normoxia (21 kPa O_2) and once in hypoxia (12 kPa O_2), in two separate experiments that were conducted in random order and separated by at least 2 days. Breathing and metabolism were determined as described above, except that CO_2 fraction was continuously measured in dry incurrent air, and incurrent and excurrent air was dried and scrubbed free of CO_2 with soda lime and ascarite before O_2 fraction was measured. We therefore calculated $\dot{V}O_2$ using appropriate equations for dry and CO_2-free air as described by Lighton (24).

Statistical analysis. Two-factor ANOVA and Holm-Sidak post-tests were used throughout. The main effects of acclimation environment (normoxia vs. hypoxia) and inspired gas composition (repeated measure) were evaluated within each population to determine the impacts of hypoxia acclimation on O_2 or CO_2 sensitivity of breathing. Values are reported as mean ± SEM. All statistical analysis was conducted with SigmaStat software (v. 3.5) with a significance level of $P < 0.05$.

Results

Acute hypoxia responses. Hypoxia acclimation altered breathing in lowland mice, but not in highland mice (Fig. 1,2, Table 1,2). Amongst first generation mice (G1), lowlanders exhibited a robust ventilatory response to hypoxia that was primarily driven by increases in breathing frequency, offset slightly by small decreases in tidal volume (Fig. 1A,C,E, Table 1). Hypoxia acclimation had an appreciable effect on breathing, reflected primarily by strong increases in tidal volume and reductions in breathing frequency (Fig. 1C,E, Table 1), and there was a strong trend for total ventilation to increase after hypoxia acclimation (Fig. 1A, Table 1). $\dot{V}O_2$ and body

temperature (T_b) declined in response to hypoxia challenge, but hypoxia acclimation tended to reduce these declines (Table 1,2). In contrast, hypoxia acclimation had very little effect on breathing or metabolism in G1 highland mice (Fig. 1B,D,F, Table 1,2).

The diminished effects of hypoxia acclimation in G1 highland mice were also observed in second generation (G2) highland mice (Fig. 2, Tables 1-2). Hypoxia acclimation had a strong effect on breathing in G2 lowlanders, as it did in G1 lowlanders, as reflected by significant increases in tidal volume (Fig. 2E) and reductions in breathing frequency (Fig. 2C) during acute hypoxia. Therefore, even though there was some subtle variation in the magnitude of breathing and the HVR across generations, the effects of hypoxia acclimation on breathing pattern in lowlanders was generally preserved. In contrast, hypoxia acclimation had no effect on breathing or metabolism in G2 highlanders (Fig. 2, Table 2), as was observed in G1 highlanders.

Hypercapnic ventilatory response. We next sought to determine whether hypoxia acclimation increases CO_2 sensitivity of breathing, and whether this effect of hypoxia acclimation is altered in highlanders. We examined these possibilities by measuring the ventilatory response to stepwise hypercapnia in normoxia and hypoxia. We found that hypoxia acclimation enhanced the hypercapnic ventilatory response in both lowland and highland mice when tested in normoxic conditions (Fig. 3, Table 3). Both lowlanders and highlanders exhibited similar robust ventilatory responses to increasing CO_2 that were driven by increases in both tidal volume and breathing frequency (Fig. 3, Table 3). This response was augmented similarly after hypoxia acclimation in both populations, particularly at higher CO_2 levels, due to further increases in tidal volume in both populations and breathing frequency in lowlanders. $\dot{V}O_2$ and T_b

were not altered by acute hypercapnia or by hypoxia acclimation in either population (Table 3, data not shown).

We found that hypoxia acclimation resulted in comparable increases in the hypercapnic ventilatory response when it was tested in hypoxic conditions (12 kPa O_2) (Fig. 4, Table 3). The response to acute hypercapnia was similar to that observed in normoxic conditions, except that ventilation was higher overall due to hypoxia. Hypoxia acclimation augmented the hypercapnic ventilatory response measured in hypoxia in both populations, as observed for the hypercapnic ventilatory response measured in normoxia, but in this case the increases in ventilation were entirely caused by increases in tidal volume (Fig. 4E,F). These findings suggest that hypoxia acclimation increases the CO_2 sensitivity of breathing in both highland and lowland mice, but in general, there were no apparent differences in the hypercapnic ventilatory response of highlanders compared to lowlanders.

Acute hypoxia responses in 2 kPa CO_2. We sought to examine whether the apparent lack of VAH in highlanders could be a bi-product of increases in ventilatory sensitivity to respiratory hypocapnia after chronic hypoxia. Given that hypoxia acclimation appears to augment CO_2 sensitivity (Fig. 3,4), this could foreseeably augment the restraining influence of respiratory hypocapnia on the poikilocapnic HVR, and thus offset other effects of chronic hypoxia that stimulate breathing and would tend to cause VAH. We examined this possibility by measuring the HVR during moderately elevated inspired CO_2 (2 kPa CO_2) to offset respiratory hypocapnia, with the prediction that increases in CO_2 would amplify the effects of chronic hypoxia on breathing. There was some modest support for this prediction, as reflected by the apparent increase in the magnitude of the effects of hypoxia acclimation on average in both populations

(compare the variation in tidal volume in Fig. 5 to Fig. 1). In highlanders in particular, hypoxia acclimation increased tidal volume at the higher PO_2s when measured in the presence of 2 kPa CO_2 (Fig. 5F, Table 4). However, hypoxia acclimation still had a much smaller effect on breathing in highlanders than in lowlanders, and there were still no significant main effects of hypoxia acclimation on total ventilation, breathing frequency, or tidal volume in highlanders (Table 4). Otherwise the effects of acute hypoxia on breathing and metabolism in the presence of 2 kPa CO_2 were quite similar to those observed without CO_2 in the inspired gas (Table 4,5). Therefore, the apparent lack of VAH in highlanders cannot be explained by variation in the CO_2 sensitivity of breathing.

DISCUSSION

Effective control of ventilation is critically important for small endotherms in the O_2-limited environment at high altitude, in order to maintain adequate tissue O_2 supply for thermogenesis and exercise. Previously, we observed that hypoxia acclimation had little effect on breathing and the HVR of high-altitude deer mice, at levels of chronic hypoxia that did induce a VAH response in lowland mice (16). Here, we show that the apparent blunting of VAH is observed across multiple generations of lab-raised highland mice, suggesting that this blunting has evolved in response to the challenges of life at high altitude. This blunting was not associated with any evolved change in the effects of hypoxia acclimation on CO_2 sensitivity of breathing in highland mice. As a consequence, variation in the ventilatory sensitivity to respiratory hypocapnia does not appear to contribute to the attenuation of VAH in highlanders.

VAH is attenuated in high-altitude deer mice. Our findings suggest that the apparent lack of VAH in high-altitude deer mice results from an evolved change in the magnitude of hypoxia-induced plasticity of breathing. In previous studies, it has been challenging to establish whether changes in the control of breathing in highland taxa are evolved and genetically based, because it has often been difficult to exclude the influence of developmental and/or parental exposure to different environments (1, 31). The blunted VAH we previously reported in first-generation highlanders raised in captivity suggested that this blunting cannot be explained by differences in developmental environment (16). However, it was possible that these differences between populations of first-generation mice could be explained by exposure of parents and/or germ cells to different environments. For example, exposure of parents and their germline cells to hypoxia has persistent effects on hypoxia tolerance in offspring in zebrafish (13). However, the persistent lack of VAH in the second-generation of highlanders raised in captivity cannot be attributed to the exposure of parents and germline cells to the high-altitude environment, and the attenuation of VAH is more likely to be an evolved trait.

What are the mechanisms that account for blunting of the VAH in high-altitude deer mice? In lowlanders, VAH arises from adjustments in the carotid bodies and the central nervous system. Chronic hypoxia enhances O_2 chemosensitivity of the carotid bodies, which appears to be associated with neovascularization and growth of the organ, and changes in O_2 signalling by O_2-sensitive glomus cells (20, 42, 57). Some of these adjustments appear to be attenuated in highland deer mice, as reflected by our previous observation that highlanders do not exhibit carotid body growth in response to chronic hypoxia (16). Chronic hypoxia also leads to increases in central gain of the afferent signals from the carotid body in lowlanders (e.g. changes in glutamatergic signalling in the NTS) (35, 43), and it is possible that these mechanisms are also

attenuated in highland mice. However, before carrying out the current study, we could not exclude the possibility that the apparent blunting in VAH arose from variation in the effects of CO_2 on breathing, because the HVR was measured under poikilocapnic conditions. Our results here suggest that this is not the case, because highlanders still exhibited a blunted VAH when respiratory hypocapnia was alleviated by exposure to moderately elevated inspired CO_2 (Fig. 5). This likely implies that VAH and its underlying peripheral and/or central mechanisms are indeed blunted in highland mice.

Effects of chronic hypoxia on the CO_2 sensitivity of breathing. Hypoxia acclimation increased CO_2 sensitivity of breathing, driven primarily by larger increases in tidal volume in response to high CO_2. This observation is consistent with previous observations in humans, in which chronic hypoxia increases ventilatory CO_2 sensitivity and/or lowers the recruitment threshold above which blood CO_2 stimulates ventilation (7, 8, 49). Chronic hypoxia is well known to induce mechanisms of acid-base compensation to counter respiratory hypocapnia and alkalosis (40), so it is possible that apparent changes in the CO_2 sensitivity of breathing arise from changes in the relationship between CO_2 and pH or in pH buffering of the blood (7, 8). It is also possible that increases in ventilatory CO_2 sensitivity in response to chronic hypoxia arise from increases in the chemosensitivity of peripheral or central CO_2/pH chemoreceptors, as suggested by previous studies in humans (9). If this is also the case in deer mice, then the mechanisms likely do not depend upon hypoxia-induced growth of the carotid bodies, which occurs in lowlanders but not in highlanders (16).

Ventilatory sensitivity to CO_2 appeared to be similar in in high-altitude mice and their low-altitude counterparts, both before and after hypoxia acclimation. This contrasts previous

findings in some other high-altitude taxa. Ventilatory sensitivity to CO_2 and ventilatory recruitment threshold are lower in Himalayans residing at high-altitude than in lowlanders at sea-level (50). Similarly, ventilatory sensitivity to CO_2 is lower in Andeans residing at high altitude than in lowlanders acclimatized to the same altitude for 10 days (49). In bar-headed geese, a species that flies over the Himalayas during its biannual migration, ventilatory sensitivity to hypercapnia is unaltered but sensitivity to respiratory hypocapnia is reduced, such that bar-headed geese breathe more than low-altitude birds when exposed to poikilocapnic hypoxia (48). Therefore, there appears to be differences across highland taxa, with ventilatory sensitivity to CO_2 having been either unaltered or reduced.

High-altitude adaptation and control of breathing. The emerging evidence suggests that there are a number of changes in the control of breathing by hypoxia in high-altitude deer mice. Our results here and in previous studies suggest that VAH and hypoxia-induced growth of the carotid bodies are attenuated in highlanders, which may represent an evolved loss of plasticity associated with high-altitude adaptation. Highlanders instead exhibit a fixed breathing pattern, characterized by deep but less frequent breaths, which should improve effective (alveolar) ventilation, and thus help increase arterial O_2 saturation in hypoxia (16). These changes exist without any apparent alterations in ventilatory CO_2 sensitivity. An intriguing question to consider is why these evolved changes have taken place? VAH increases ventilation and thus improves respiratory gas exchange, so why have high-altitude mice not maintained the VAH response that is typical of lowlanders? One possibility is that highland mice have undergone the evolutionary process of genetic assimilation, in which a phenotype that originally exhibits adaptive plasticity becomes genetically fixed (assimilated) (6, 23, 38). Another possibility that

we and others have discussed previously is that there may have been an overall restructuring of the hypoxic chemoreflex in high-altitude deer mice (16, 29). It is possible that by fixing a breathing pattern that is beneficial for O_2 uptake, highlanders may avoid some costs associated with plasticity in response to chronic hypoxia at high altitude (e.g., chronic sympathetic activation, etc.). Given the harshness of high-altitude environments and the correspondingly strong selection favouring respiratory performance (12), evolved changes in control of breathing may help safeguard O_2 uptake and contribute to the success and high abundance of deer mice in high-altitude environments.

ACKNOWELDGEMENTS

The equipment and operational costs of this research was supported by funds from McMaster University, the Canadian Foundation for Innovation, the Ontario Ministry of Research and Innovation, and a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to G.R.S. C.M.I. was supported by a NSERC Postgraduate Scholarship and an Ontario Graduate Scholarship. G.R.S. is supported by the Canada Research Chairs Program.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: C.M.I. and G.R.S. conceived and designed the research; C.M.I. performed the experiments, analyzed the data, and wrote the first draft of the manuscript; C.M.I. and G.R.S. interpreted the results of experiments, prepared figures, and edited and revised the manuscript. Both authors approve the final version of manuscript.

REFERENCES

1. **Brutsaert T**. Why are high altitude natives so strong at high altitude? Nature vs. nurture: Genetic factors vs. growth and development. In: *Advances in Experimental Medicine and Biology*. 2016, p. 101–112.
2. **Cheviron ZA**, **Bachman GC**, **Connaty AD**, **McClelland GB**, **Storz JF**. Regulatory changes contribute to the adaptive enhancement of thermogenic capacity in high-altitude deer mice. *Proc Natl Acad Sci* 109: 8635–8640, 2012.
3. **Cheviron ZA**, **Bachman GC**, **Storz JF**. Contributions of phenotypic plasticity to differences in thermogenic performance between highland and lowland deer mice. *J Exp Biol* 216: 1160–1166, 2013.
4. **Dawson NJ**, **Lyons SA**, **Henry DA**, **Scott GR**. Effects of chronic hypoxia on diaphragm function in deer mice native to high altitude. *Acta Physiol* 223, 2018.
5. **Drorbaugh JE**, **Fenn WO**. A barometric method for measuring ventilation in newborn infants. *Pediatrics* 16: 81–87, 1955.
6. **Ehrenreich IM**, **Pfennig DW**. Genetic assimilation: A review of its potential proximate causes and evolutionary consequences. *Ann. Bot.* 117: 769–779, 2016.
7. **Fan J-L**, **Burgess KR**, **Basnyat R**, **Thomas KN**, **Peebles KC**, **Lucas SJE**, **Lucas R a I**, **Donnelly J**, **Cotter JD**, **Ainslie PN**. Influence of high altitude on cerebrovascular and ventilatory responsiveness to CO_2. *J Physiol* 588: 539–549, 2010.
8. **Fan J-L**, **Subudhi AW**, **Evero O**, **Bourdillon N**, **Kayser B**, **Lovering AT**, **Roach RC**. AltitudeOmics: enhanced cerebrovascular reactivity and ventilatory response to CO_2 with high-altitude acclimatization and reexposure. *J Appl Physiol* 116: 911–8, 2014.
9. **Fatemian M**, **Robbins PA**. Physiological and Genomic Consequences of Intermittent Hypoxia Selected Contribution: Chemoreflex responses to CO_2 before and after an 8-h exposure to hypoxia in humans. *J Appl Physiol* 90: 1607–1614, 2001.
10. **Gonzalez C**, **Almaraz L**, **Obeso A**, **Rigual R**. Carotid body chemoreceptors: from natural stimuli to sensory discharges. *Physiol Rev* 74: 829–898, 1994.
11. **Hayes JP**. Field and maximal metabolic rates of deer mice (Peromyscus maniculatus) at low and high altitudes. *Physiol Zool* 62: 732–744, 1989.
12. **Hayes JP**, **O'Connor CS**. Natural Selection on Thermogenic Capacity of High-Altitude Deer Mice. *Evolution (N Y)* 53: 1280, 1999.
13. **Ho DH**, **Burggren WW**. Parental hypoxic exposure confers offspring hypoxia resistance in zebrafish (Danio rerio). *J Exp Biol* 215: 4208–4216, 2012.
14. **Hock R**. Physiological responses of deer mice to various native altitudes. In: *The Physiological Effects of High Altitude*, edited by Weihe W. New York: Macmillan, 1964, p. 59–70.

15. **Ivy CM**, **Scott GR**. Control of breathing and the circulation in high-altitude mammals and birds. *Comp Biochem Physiol -Part A Mol Integr Physiol* 186: 66–74, 2015.
16. **Ivy CM**, **Scott GR**. Control of breathing and ventilatory acclimatization to hypoxia in deer mice native to high altitudes. *Acta Physiol* 221: 266–282, 2017.
17. **Ivy CM**, **Scott GR**. Ventilatory acclimatization to hypoxia in mice: Methodological considerations. *Respir Physiol Neurobiol* 235: 95–103, 2017.
18. **Ivy CM**, **York JM**, **Lague SL**, **Chua BA**, **Alza L**, **McCracken KG**, **Milsom WK**, **Scott GR**. Validation of a pulse oximetry system for high-altitude waterfowl by examining the hypoxia responses of the Andean goose (Chloephaga melanoptera). *Physiol Biochem Zool* 91: 859–867, 2018.
19. **Jacky JP**. Barometric measurement of tidal volume: effects of pattern and nasal temperature. *J Appl Physiol* 49: 319–325, 1980.
20. **Kusakabe T**, **Powell FL**, **Ellisman MH**. Ultrastructure of the glomus cells in the carotid body of chronically hypoxic rats: With special reference to the similarity of amphibian glomus cells. *Anat Rec* 237: 220–227, 1993.
21. **Lague SL**, **Chua B**, **Alza L**, **Scott GR**, **Frappell PB**, **Zhong Y**, **Farrell AP**, **McCracken KG**, **Wang Y**, **Milsom WK**. Divergent respiratory and cardiovascular responses to hypoxia in bar-headed geese and Andean birds. *J Exp Biol* 220: 4186–4194, 2017.
22. **Lague SL**, **Chua B**, **Farrell AP**, **Wang Y**, **Milsom WK**. Altitude matters: differences in cardiovascular and respiratory responses to hypoxia in bar-headed geese reared at high and low altitudes. *J Exp Biol* 219: 1974–1984, 2016.
23. **Lande R**. Evolution of phenotypic plasticity in colonizing species. *Mol Ecol* 24: 2038–2045, 2015.
24. **Lighton JRB**. Measuring Metabolic Rates : A Manual for Scientists. : 1–200, 2008.
25. **Lui M a**, **Mahalingam S**, **Patel P**, **Connaty AD**, **Ivy CM**, **Cheviron Z a**, **Storz JF**, **McClelland GB**, **Scott GR**. High-altitude ancestry and hypoxia acclimation have distinct effects on exercise capacity and muscle phenotype in deer mice. *Am J Physiol Regul Integr Comp Physiol* 308: R779–R791, 2015.
26. **Mahalingam S**, **McClelland GB**, **Scott GR**. Evolved changes in the intracellular distribution and physiology of muscle mitochondria in high-altitude native deer mice. *J Physiol* 595: 4785–4801, 2017.
27. **Mathew L**, **Gopinath PM**, **Purkayastha SS**, **Sen Gupta J**, **Nayar HS**. Chemoreceptor sensitivity in adaptation to high altitude. *Aviat Sp Environ Med* 54: 121–126, 1983.
28. **McClelland GB**, **Hochachka PW**, **Weber JM**. Carbohydrate utilization during exercise after high-altitude acclimation: a new perspective. *Proc Natl Acad Sci U S A* 95: 10288–10293, 1998.
29. **Milsom WK**. Different solutions to restoring oxygen delivery at altitude. *Acta Physiol.* 222: 2018.
30. **Monge C**, **Leon-Velarde F**. Physiological Adaptation to High Altitude : Oxygen Transport in Mammals and Birds. *Physiol Rev* 71: 1135–1172, 1991.
31. **Moore LG**. Measuring high-altitude adaptation. *J Appl Physiol* 123: 1371–1385, 2017.
32. **Moore LG**, **Harrison GL**, **McCullough RE**, **McCullough RG**, **Micco a J**, **Tucker a**, **Weil J V**, **Reeves JT**. Low acute hypoxic ventilatory response and hypoxic depression in acute altitude sickness. *J Appl Physiol* 60: 1407–1412, 1986.
33. **Natarajan C**, **Inoguchi N**, **Weber RE**, **Fago A**, **Moriyama H**, **Storz JF**. Epistasis among adaptive mutations in deer mouse hemoglobin. *Science (80-)* 340: 1324–1327,

2013.
34. **Nikel KE**, **Shanishchara NK**, **Ivy CM**, **Dawson NJ**, **Scott GR**. Effects of hypoxia at different life stages on locomotory muscle phenotype in deer mice native to high altitudes. *Comp Biochem Physiol Part - B Biochem Mol Biol* : 0–1, 2017.
35. **Pamenter ME**, **Carr JA**, **Go A**, **Fu Z**, **Reid SG**, **Powell FL**. Glutamate receptors in the nucleus tractus solitarius contribute to ventilatory acclimatization to hypoxia in rat. *J Physiol* 592: 1839–56, 2014.
36. **Pardal R**, **Ortega-Sáenz P**, **Durán R**, **López-Barneo J**. Glia-like Stem Cells Sustain Physiologic Neurogenesis in the Adult Mammalian Carotid Body. *Cell* 131: 364–377, 2007.
37. **Pichon A**, **Zhenzhong B**, **Favret F**, **Jin G**, **Shufeng H**, **Marchant D**, **Richalet J-P**, **Ge R-L**. Long-term ventilatory adaptation and ventilatory response to hypoxia in plateau pika (Ochotona curzoniae): role of nNOS and dopamine. *Am J Physiol Regul Integr Comp Physiol* 297: R978–R987, 2009.
38. **Pigliucci M**. Phenotypic plasticity and evolution by genetic assimilation. *J Exp Biol* 209: 2362–2367, 2006.
39. **Powell FL**. The influence of chronic hypoxia upon chemoreception. *Respir Physiol Neurobiol* 157: 154–161, 2008.
40. **Powell FL**, **Fedde MR**, **Gratz RK**, **Scheid P**. Ventilatory response to CO_2 in birds. I. Measurements in the unanesthetized duck. *Respir Physiol* 35: 349–359, 1978.
41. **Powell FL**, **Milsom WK**, **Mitchell GS**. Time domains of the hypoxic ventilatory response. *Respir Physiol* 112: 123–134, 1998.
42. **Prabhakar NR**, **Jacono FJ**. Cellular and molecular mechanisms associated with carotid body adaptations to chronic hypoxia. *High Alt Med Biol* 6: 112–120, 2005.
43. **Reid SG**, **Powell FL**. Effects of chronic hypoxia on MK-801-induced changes in the acute hypoxic ventilatory response. *J Appl Physiol* 99: 2108–2114, 2005.
44. **Schoene RB**, **Roach RC**, **Hackett PH**, **Sutton JR**, **Cymerman A**, **Houston CS**. Operation everest II: Ventilatory adaptation during gradual decompression to extreme altitude. *Med Sci Sports Exerc* 22: 804–810, 1990.
45. **Schwenke DO**, **Bolter CP**, **Cragg PA**. Are the carotid bodies of the guinea-pig functional? *Comp Biochem Physiol Part A Mol Integr Physiol* 146: 180–188, 2007.
46. **Scott GR**, **Elogio TS**, **Lui MA**, **Storz JF**, **Cheviron ZA**. Adaptive Modifications of Muscle Phenotype in High-Altitude Deer Mice Are Associated with Evolved Changes in Gene Regulation. *Mol Biol Evol* 32: 1962–1976, 2015.
47. **Scott GR**, **Milsom WK**. Flying high: A theoretical analysis of the factors limiting exercise performance in birds at altitude. *Respir Physiol Neurobiol* 154: 284–301, 2006.
48. **Scott GR**, **Milsom WK**. Control of breathing and adaptation to high altitude in the bar-headed goose. *Am J Physiol Regul Integr Comp Physiol* 293: 379–391, 2007.
49. **Slessarev M**, **Mardimae A**, **Preiss D**, **Vesely A**, **Balaban DY**, **Greene R**, **Duffin J**, **Fisher JA**. Differences in the control of breathing between Andean highlanders and lowlanders after 10 days acclimatization at 3850 m. *J Physiol* 588: 1607–1621, 2010.
50. **Slessarev M**, **Prisman E**, **Ito S**, **Watson RR**, **Jensen D**, **Preiss D**, **Greene R**, **Norboo T**, **Stobdan T**, **Diskit D**, **Norboo A**, **Kunzang M**, **Appenzeller O**, **Duffin J**, **Fisher JA**. Differences in the control of breathing between Himalayan and sea-level residents. *J Physiol* 588: 1591–1606, 2010.
51. **Snyder LRG**, **Born S**, **Lechner AJ**. Blood oxygen affinity in high- and low-altitude

populations of the deer mouse. *Respir Physiol* 48: 89–105, 1982.
52. **Somogyi RB**, **Preiss D**, **Vesely A**, **Fisher JA**, **Duffin J**. Changes in respiratory control after 5 days at altitude. *Respir Physiol Neurobiol* 145: 41–52, 2005.
53. **Storz JF**, **Runck AM**, **Sabatino SJ**, **Kelly JK**, **Ferrand N**, **Moriyama H**, **Weber RE**, **Fago A**. Evolutionary and functional insights into the mechanism underlying high-altitude adaptation of deer mouse hemoglobin. *Proc Natl Acad Sci U S A* 106: 14450–14455, 2009.
54. **Storz JF**, **Scott GR**, **Cheviron ZA**. Phenotypic plasticity and genetic adaptation to high-altitude hypoxia in vertebrates. *J Exp Biol* 213: 4125–4136, 2010.
55. **Tate KB**, **Ivy CM**, **Velotta JP**, **Storz JF**, **McClelland GB**, **Cheviron ZA**, **Scott GR**. Circulatory mechanisms underlying adaptive increases in thermogenic capacity in high-altitude deer mice. *J Exp Biol* 220: 3616–3620, 2017.
56. **Velotta JP**, **Ivy CM**, **Wolf CJ**, **Scott GR**, **Cheviron ZA**. Maladaptive phenotypic plasticity in cardiac muscle is attenuated in high-altitude deer mice. *Evolution (N Y)* , [date unknown].
57. **Wang Z-Y**, **Olson EB**, **Bjorling DE**, **Mitchell GS**, **Bisgard GE**. Sustained hypoxia-induced proliferation of carotid body type I cells in rats. *J Appl Physiol* 104: 803–808, 2008.

FIGURE LEGENDS

Fig. 1. Hypoxia acclimation has very little effect on breathing in highland deer mice from the first generation (G1) raised in captivity, unlike G1 mice from low altitude. * Significant pairwise difference between acclimation (acc.) groups within each PO_2 using Holm-Sidak post-tests (n as follows:13 normoxia-acclimated lowlanders, 15 hypoxia-acclimated lowlanders, 15 normoxia-acclimated highlanders, 13 hypoxia-acclimated highlanders).

Fig. 2. Hypoxia acclimation has no effect on breathing in highland deer mice from the second-generation (G2) raised in captivity, unlike G2 mice from low altitude. * Significant pairwise difference between acclimation (acc.) groups within each PO_2 using Holm-Sidak post-tests (n as follows: 10 normoxia-acclimated lowlanders, 9 hypoxia-acclimated lowlanders, 14 normoxia-acclimated highlanders, 14 hypoxia-acclimated highlanders).

Fig. 3. Hypoxia acclimation increased ventilatory sensitivity to CO_2 in both lowland and highland mice, when measured in normoxic conditions (21 kPa O_2). * Significant pairwise difference between acclimation groups within each PCO_2 using Holm-Sidak post-tests (n as follows: 10 normoxia-acclimated lowlanders, 9 hypoxia-acclimated lowlanders, 13 normoxia-acclimated highlanders, 14 hypoxia-acclimated highlanders).

Fig. 4. Hypoxia acclimation increased ventilatory sensitivity to CO_2 in both lowland and highland mice, when measured in hypoxic conditions (12 kPa O_2). * Significant pairwise difference between acclimation groups within each PCO_2 using Holm-Sidak post-tests (n as in Fig. 3).

Fig. 5. Hypoxic ventilatory responses measured in the presence of moderately elevated levels of inspired CO_2 (2 kPa). * Significant pairwise difference between acclimation groups within each PO_2 using Holm-Sidak post-tests (n as in Fig. 1).

Table 1. *Statistical results of two-way ANOVA of acute hypoxia responses*

		Acclimation Environment		Acute PO_2		Interaction	
		F	P	F	P	F	P
First Generation Mice							
Total	Lowlander	2.612	0.118	78.61	**<0.001**	1.889	0.101
Ventilation	Highlander	1.149	0.294	74.62	**<0.001**	0.416	0.837
Breathing	Lowlander	11.67	**0.002**	179.9	**<0.001**	12.07	**<0.001**
Frequency	Highlander	1.160	0.291	286.4	**<0.001**	3.841	**0.003**
Tidal	Lowlander	18.74	**<0.001**	10.12	**<0.001**	0.555	0.735
Volume	Highlander	0.092	0.764	31.17	**<0.001**	0.954	0.449
O_2	Lowlander	1.233	0.277	12.4	**<0.001**	1.989	0.084
Consumption	Highlander	0.794	0.381	22.92	**<0.001**	2.148	0.064
Body	Lowlander	6.047	**0.021**	196.8	**<0.001**	8.878	**0.006**
Temperature	Highlander	12.92	**0.001**	61.55	**<0.001**	6.532	**0.017**
Second Generation Mice							
Total	Lowlander	1.418	0.251	17.78	**<0.001**	2.212	0.061
Ventilation	Highlander	0.043	0.838	24.67	**<0.001**	3.467	**0.006**
Breathing	Lowlander	5.799	**0.028**	59.74	**<0.001**	5.089	**<0.001**
Frequency	Highlander	0.486	0.492	236.6	**<0.001**	1.111	0.358
Tidal	Lowlander	4.830	**0.043**	6.258	**<0.001**	6.296	**<0.001**
Volume	Highlander	0.562	0.460	71.05	**<0.001**	0.979	0.433
O_2	Lowlander	0.827	0.377	17.84	**<0.001**	0.945	0.457
Consumption	Highlander	0.016	0.900	27.12	**<0.001**	4.900	**<0.001**
Body	Lowlander	0.057	0.815	143.0	**<0.001**	2.486	0.134
Temperature	Highlander	6.126	**0.020**	32.53	**<0.001**	4.640	**0.041**

One degree of freedom for acclimation environment, 5 degrees of freedom for acute PO_2 and their interaction, 130 degrees of freedom for the residuals of first generation lowland and highland mice and second generation highland mice, and 80 degrees of freedom for the residuals of second generation lowland mice.

547 Table 2. *The rate of O_2 consumption and body temperature during acute hypoxia exposure*

Acute PO_2 (kPa)	Lowland *P. leucopus*		Highland *P. maniculatus*	
	Normoxia Acclimated	Hypoxia Acclimated	Normoxia Acclimated	Hypoxia Acclimated
	First Generation Mice			
	O_2 Consumption Rate (ml g^{-1} min^{-1})			
21	0.045 ± 0.004	0.045 ± 0.003	0.048 ± 0.003	0.045 ± 0.003
16	0.426 ± 0.004	0.040 ± 0.002	0.039 ± 0.003	0.039 ± 0.004
12	0.039 ± 0.003	0.041 ± 0.003	0.035 ± 0.002	0.037 ± 0.002
10	0.035 ± 0.003	0.037 ± 0.002	0.032 ± 0.002	0.038 ± 0.002
9	0.031 ± 0.002	0.038 ± 0.002	0.031 ± 0.002	0.037 ± 0.002
8	0.027 ± 0.002	0.036 ± 0.002	0.030 ± 0.002	0.033 ± 0.002
	Body Temperature (°C)			
21	37.29 ± 0.29	37.25 ± 0.24	36.08 ± 0.24	36.49 ± 0.23
8	33.52 ± 0.21	34.80 ± 0.21*	33.60 ± 0.23	35.20 ± 0.34*
	Second Generation Mice			
	O_2 Consumption Rate (ml g^{-1} min^{-1})			
21	0.045 ± 0.004	0.044 ± 0.004	0.061 ± 0.002	0.051 ± 0.003
16	0.040 ± 0.003	0.039 ± 0.003	0.055 ± 0.003	0.052 ± 0.003
12	0.038 ± 0.003	0.039 ± 0.004	0.051 ± 0.003	0.049 ± 0.003
10	0.032 ± 0.003	0.037 ± 0.003	0.045 ± 0.002	0.049 ± 0.004
9	0.031 ± 0.002	0.035 ± 0.003	0.039 ± 0.001	0.043 ± 0.003
8	0.028 ± 0.002	0.031 ± 0.002	0.037 ± 0.001	0.042 ± 0.002
	Body Temperature (°C)			
21	37.63 ± 0.33	37.45 ± 0.22	36.23 ± 0.29	36.58 ± 0.30
8	34.30 ± 0.19	34.70 ± 0.30	34.50 ± 0.23	35.80 ± 0.31*

548 PO_2, partial pressure of O_2; values are mean ± SEM.
549 * Significant pairwise difference from normoxia acclimated mice of the same population.
550
551
552

Table 3. *Statistical results of two-way ANOVA of acute hypercapnia responses, measured in normoxia or hypoxia*

		Acclimation Environment		Acute PCO_2		Interaction	
		F	P	F	P	F	P
Normoxic Conditions (21 kPa O_2)							
Total	Lowlander	7.437	**0.014**	92.07	**<0.001**	9.496	**<0.001**
Ventilation	Highlander	11.68	**0.002**	249.3	**<0.001**	20.26	**<0.001**
Breathing	Lowlander	2.535	0.130	51.60	**<0.001**	3.006	**0.039**
Frequency	Highlander	0.223	0.641	181.2	**<0.001**	3.477	**0.020**
Tidal	Lowlander	3.451	0.081	106.0	**<0.001**	5.843	**0.002**
Volume	Highlander	12.55	**0.002**	120.8	**<0.001**	15.61	**<0.001**
O_2	Lowlander	3.022	0.100	1.741	0.170	0.755	0.524
Consumption	Highlander	0.579	0.454	1.404	0.248	0.315	0.815
Body	Lowlander	0.045	0.835	5.147	**0.037**	0.572	0.460
Temperature	Highlander	0.055	0.817	39.52	**<0.001**	0.374	0.546
Hypoxic Conditions (12 kPa O_2)							
Total	Lowlander	9.610	**0.007**	87.13	**<0.001**	11.52	**<0.001**
Ventilation	Highlander	4.372	**0.047**	118.6	**<0.001**	4.831	**0.004**
Breathing	Lowlander	0.088	0.770	16.40	**<0.001**	8.088	**<0.001**
Frequency	Highlander	1.925	0.178	45.09	**<0.001**	0.723	0.541
Tidal	Lowlander	4.916	**0.041**	195.6	**<0.001**	5.279	**0.003**
Volume	Highlander	14.87	**<0.001**	210.6	**<0.001**	14.00	**<0.001**
O_2	Lowlander	0.001	0.991	1.020	0.392	1.262	0.297
Consumption	Highlander	0.095	0.761	3.722	**0.015**	1.835	0.148
Body	Lowlander	1.087	0.312	9.683	**0.006**	0.184	0.673
Temperature	Highlander	0.444	0.511	24.77	**<0.001**	0.776	0.387

One degree of freedom for acclimation environment, 3 degrees of freedom for acute PCO_2 and their interaction, 51 and 75 degrees of freedom for the residuals of lowland and highland mice, respectively, in normoxic and hypoxic conditions.

Table 4. *Statistical results of two-way ANOVA of acute hypoxia responses, measured with elevated inspired CO_2*

		Acclimation Environment		Acute PO_2		Interaction	
		F	P	F	P	F	P
Total	Lowlander	4.082	0.054	41.97	**<0.001**	0.451	0.812
Ventilation	Highlander	1.672	0.207	37.58	**<0.001**	0.800	0.552
Breathing	Lowlander	10.09	**0.004**	93.50	**<0.001**	10.64	**<0.001**
Frequency	Highlander	0.001	0.987	130.7	**<0.001**	0.428	0.828
Tidal	Lowlander	17.93	**<0.001**	1.419	0.222	1.454	0.209
Volume	Highlander	3.223	0.084	11.74	**<0.001**	1.422	0.220
O_2	Lowlander	0.656	0.425	1.226	0.300	0.237	0.946
Consumption	Highlander	3.637	0.068	10.99	**<0.001**	0.898	0.484
Body	Lowlander	7.929	**0.009**	97.30	**<0.001**	0.244	0.625
Temperature	Highlander	7.939	**0.009**	36.55	**<0.001**	8.415	**0.007**

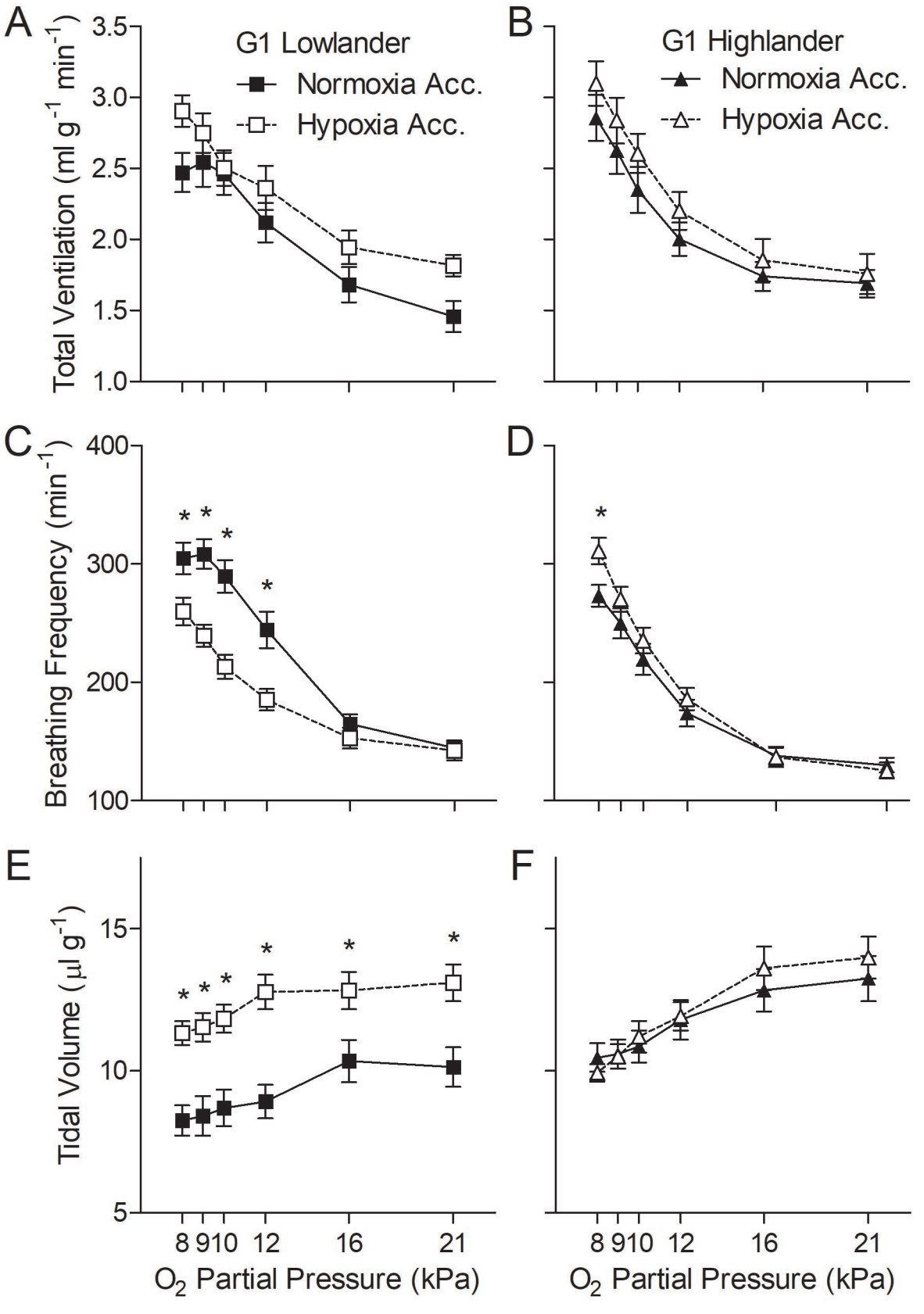
One degree of freedom for acclimation environment, 5 degrees of freedom for acute PO_2 and their interaction, 130 degrees of freedom for the residuals of lowland and highland mice.

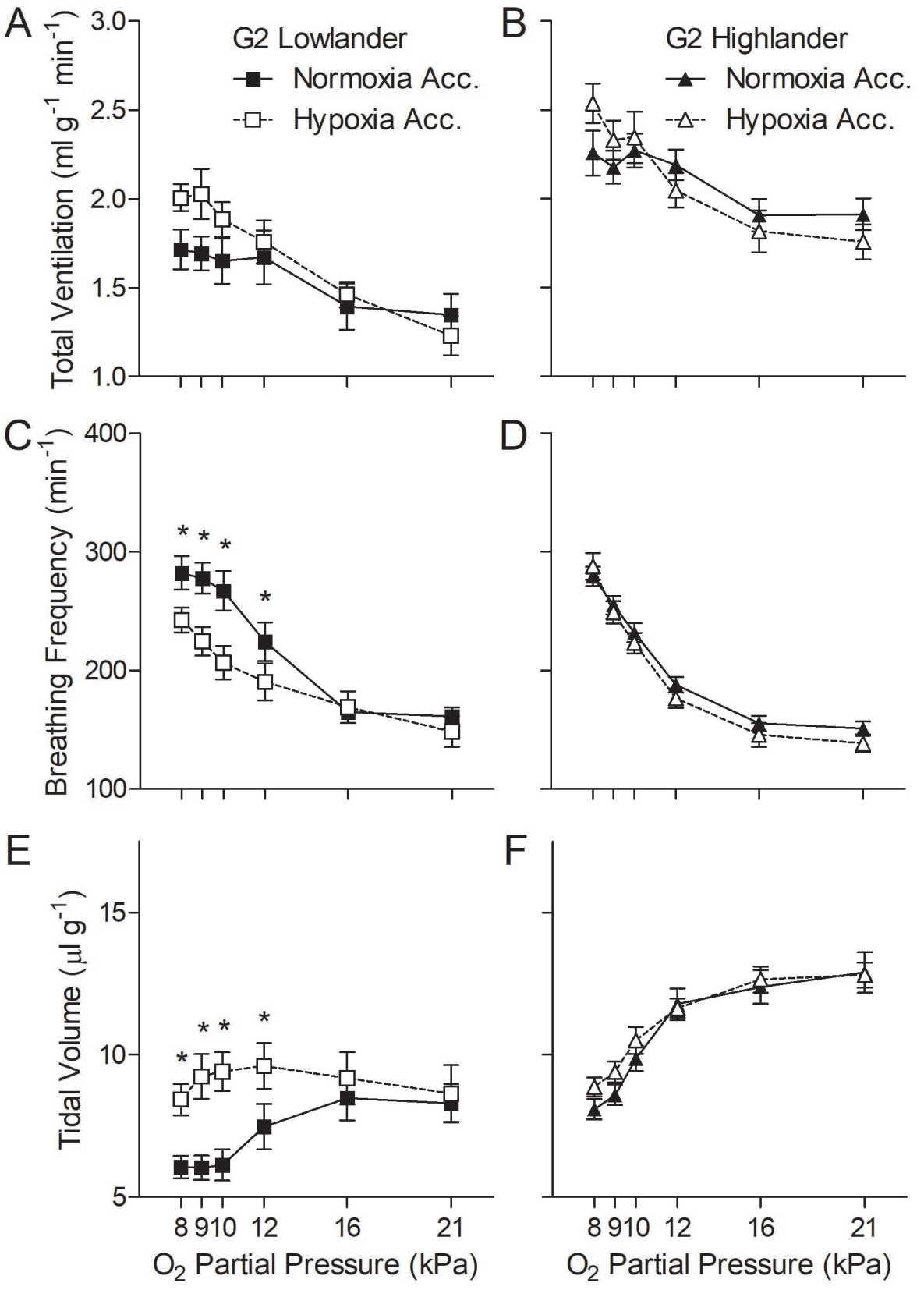
Table 5. *The rate of O_2 consumption and body temperature during acute hypoxia exposure, measured with elevated inspired CO_2*

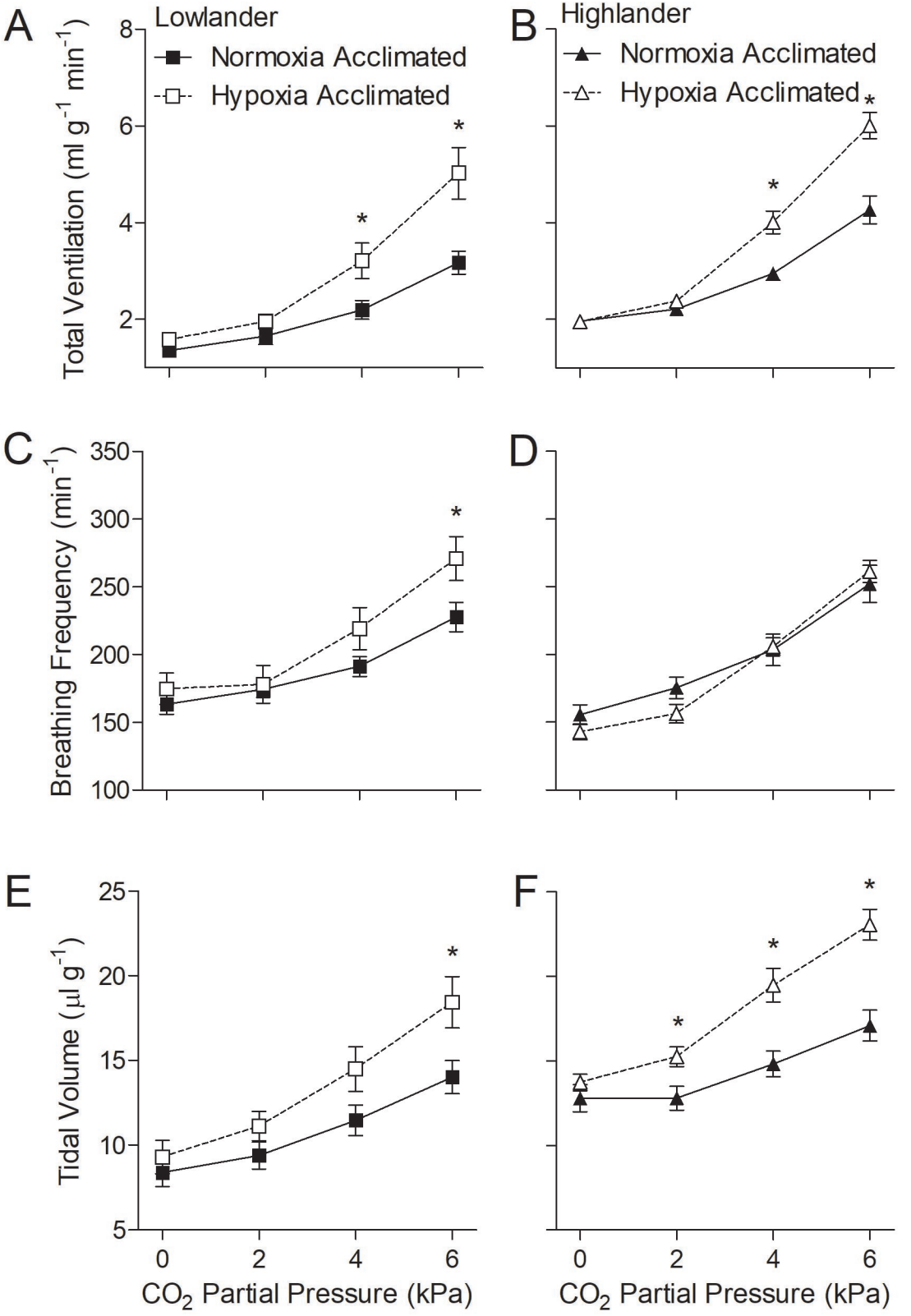
	Lowland *P. leucopus*		Highland *P. maniculatus*	
Acute PO_2 (kPa)	Normoxia Acclimated	Hypoxia Acclimated	Normoxia Acclimated	Hypoxia Acclimated
	O_2 Consumption Rate (ml g^{-1} min^{-1})			
21	0.039 ± 0.003	0.041 ± 0.003	0.052 ± 0.005	0.054 ± 0.003
16	0.037 ± 0.003	0.038 ± 0.002	0.042 ± 0.003	0.046 ± 0.002
12	0.037 ± 0.002	0.041 ± 0.002	0.039 ± 0.003	0.048 ± 0.003
10	0.039 ± 0.003	0.039 ± 0.002	0.039 ± 0.002	0.046 ± 0.003
9	0.037 ± 0.002	0.040 ± 0.002	0.039 ± 0.002	0.047 ± 0.004
8	0.035 ± 0.002	0.038 ± 0.002	0.036 ± 0.002	0.044 ± 0.004
	Body Temperature (°C)			
21	36.94 ± 0.35	37.92 ± 0.28	36.49 ± 0.22	36.36 ± 0.17
8	34.11 ± 0.35	34.79 ± 0.24	34.30 ± 0.25	35.60 ± 0.23*

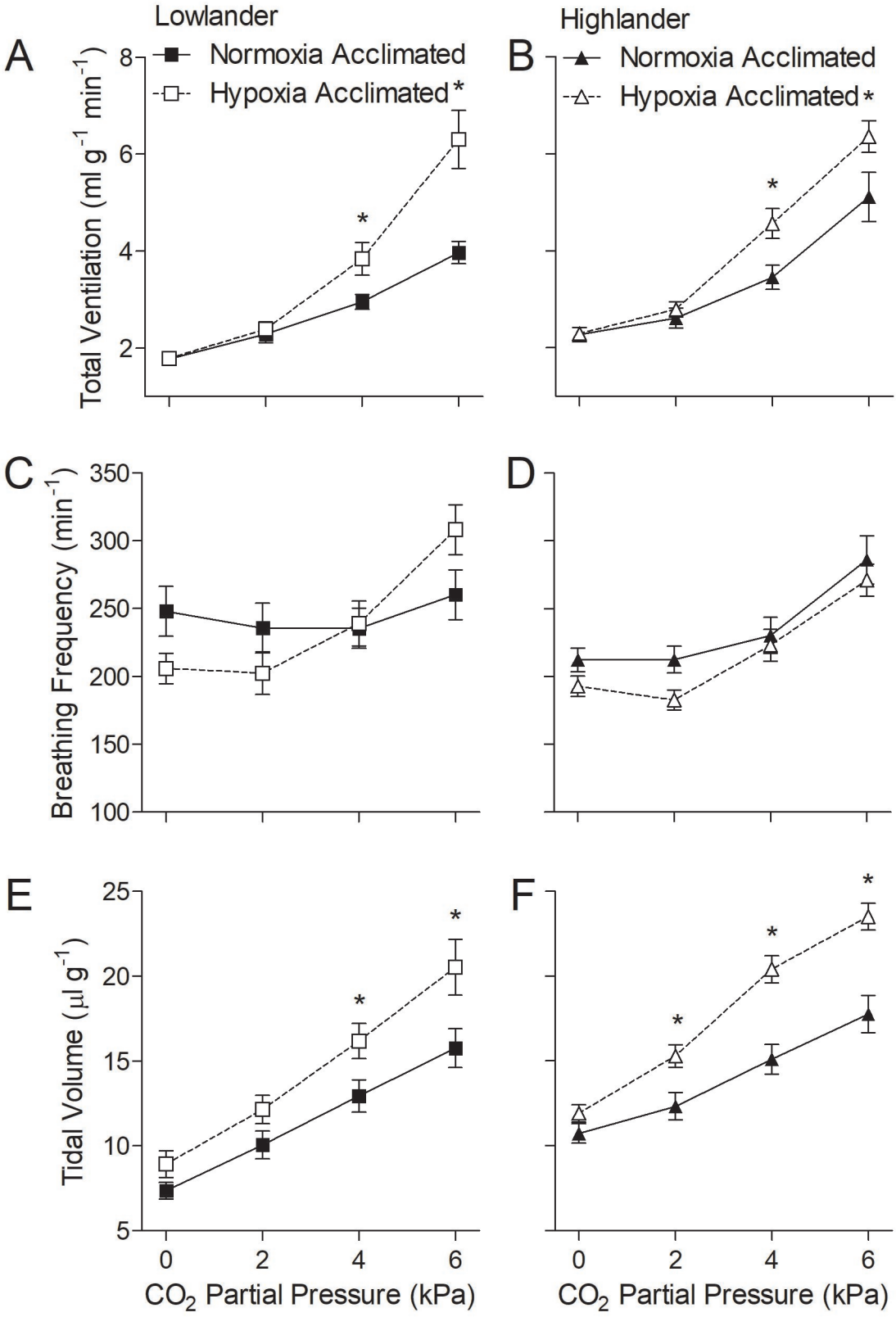
PO_2, partial pressure of O_2; values are mean ± SEM.

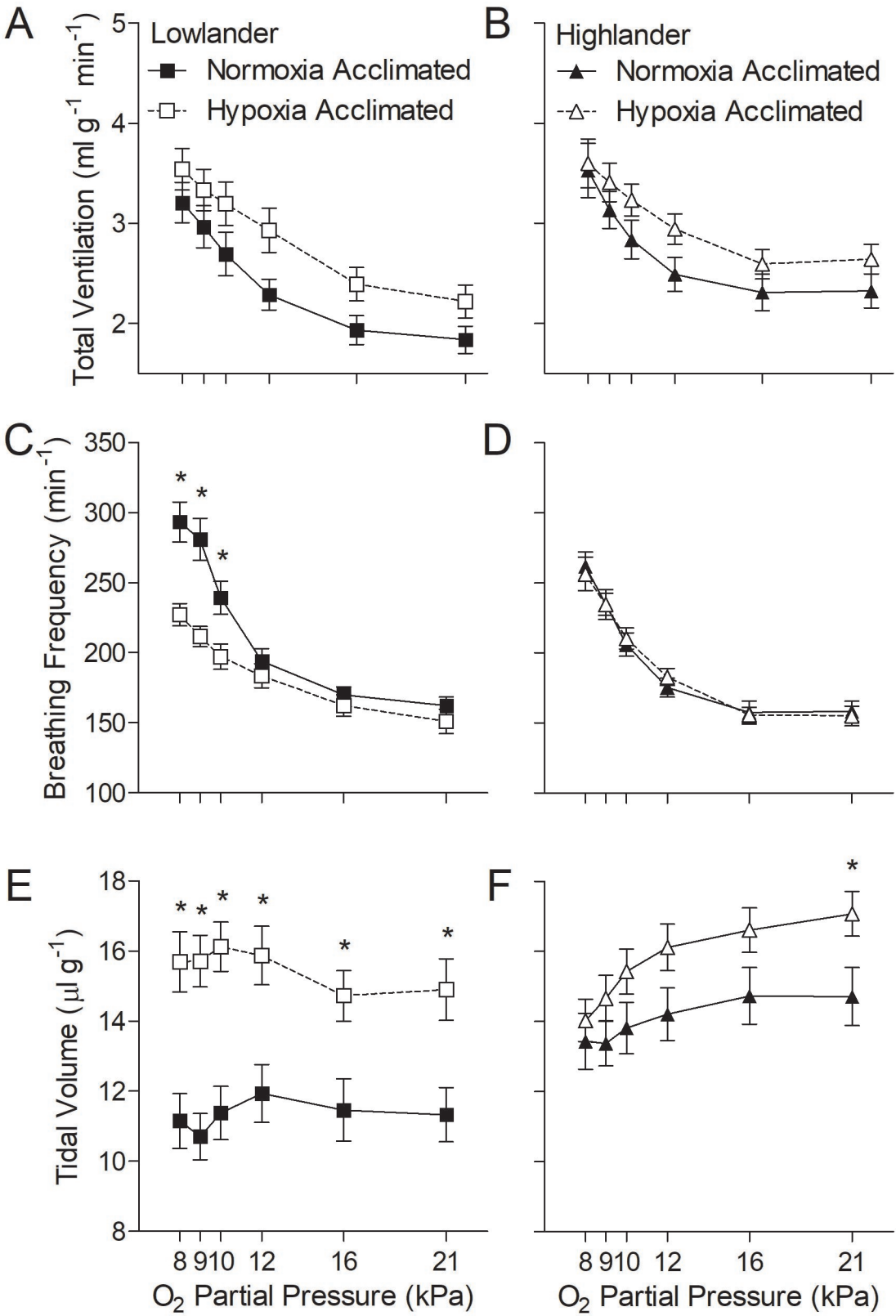
* Significant pairwise difference from normoxia acclimated mice of the same population


A
G1 Lowlander
Normoxia Acc.
Hypoxia Acc.
Total Ventilation (ml g-1 min-1)
3.5
3.0
2.5
2.0
1.5
1.0
B
G1 Highlander
Normoxia Acc.
Hypoxia Acc.
C
Breathing Frequency (min-1)
400
300
200
100
D
E
Tidal Volume (µl g-1)
15
10
5
F
8 9 10 12 16 21
O2 Partial Pressure (kPa)
8 9 10 12 16 21
O2 Partial Pressure (kPa)


A
B
C
D
E
F
G2 Lowlander
Normoxia Acc.
Hypoxia Acc.
G2 Highlander
Normoxia Acc.
Hypoxia Acc.
Total Ventilation (ml g-1 min-1)
3.0
2.5
2.0
1.5
1.0
Breathing Frequency (min-1)
400
300
200
100
Tidal Volume (µl g-1)
15
10
5
8 9 10 12 16 21
O2 Partial Pressure (kPa)
8 9 10 12 16 21
O2 Partial Pressure (kPa)


A
Lowlander
Normoxia Acclimated
Hypoxia Acclimated
Total Ventilation (ml g-1 min-1)
8
6
4
2
*
*
B
Highlander
Normoxia Acclimated
Hypoxia Acclimated
*
*
C
Breathing Frequency (min-1)
350
300
250
200
150
100
*
D
E
Tidal Volume (µl g-1)
25
20
15
10
5
*
0
2
4
6
CO2 Partial Pressure (kPa)
F
*
*
*
0
2
4
6
CO2 Partial Pressure (kPa)


Lowlander
Highlander
A
B
C
D
E
F
Normoxia Acclimated
Hypoxia Acclimated*
Total Ventilation (ml g-1 min-1)
Breathing Frequency (min-1)
Tidal Volume (µl g-1)
CO2 Partial Pressure (kPa)
CO2 Partial Pressure (kPa)


A
Lowlander
Normoxia Acclimated
Hypoxia Acclimated
Total Ventilation (ml g-1 min-1)
5
4
3
2
B
Highlander
Normoxia Acclimated
Hypoxia Acclimated
C
Breathing Frequency (min-1)
350
300
250
200
150
100
*
D
E
Tidal Volume (µl g-1)
18
16
14
12
10
8
F
8 9 10 12 16 21
O2 Partial Pressure (kPa)