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Review

## Developmental plasticity in aerobic performance in deer mice (*Peromyscus maniculatus*)<sup>☆</sup>

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### Abstract

While several studies have examined the abiotic effects of altitude (low ambient temperatures and hypoxia) on the aerobic performance of small mammals, few have explored the effects of development and maturation at different altitudes on aerobic performance as adults. We examined the basal metabolism and aerobic performance of deer mice (*Peromyscus maniculatus*) under four different developmental and testing regimes: (1) reared (gestation through weaning) and tested at high altitude; (2) reared and tested at low altitude; (3) reared at low altitude and tested at high altitude after acclimation; and (4) reared at low altitude and tested in hypoxia without acclimation. We found that mice that developed and were tested at low altitudes had a higher aerobic capacity (both aerobic performance and basal metabolic rate) than those that developed, or were acclimated as adults, at high altitudes. In addition, we found that mice that developed at high altitude did not have a higher aerobic capacity than those that developed at low altitude and were acclimated to high altitude as adults. Both groups tested at high altitudes had higher hematocrits (% red blood cells) and hemoglobin than mice tested at low altitudes. Surprisingly, mice acclimated to low altitudes and given an instantaneous exposure to hypoxia did not suffer a depression in aerobic performance.

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### 1. Introduction

One of our primary goals in physiology is to understand how performance traits adjust to variation in environmental conditions. Broadly speaking, this is accomplished in two ways: (1) phenotypic plasticity within the lifetime of individuals, and (2) genetic change across generations as

a result of natural selection (i.e. Darwinian adaptation). It is the ‘whole animal’ phenotype—a product of the interaction of these effects—that sets the limits to physiological performance. Obviously, an individual’s genetic make-up ultimately constrains physiological performance capacity, but performance may also be strongly modified within an individual’s lifetime through phenotypic plasticity in morphology (organ size), physiology (organ function), or whole body metabolic output (Daan et al., 1990; Bech and Ostnes, 1999; Koteja, 1996; Speakman and McQueenie, 1996; Hammond and Diamond, 1997; Piersma, 1998; Piersma et al., 1999; Hammond et al., 2000a,b, 2001).

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Maximal metabolic power output (aerobic capacity) during exercise or, in the case of endotherms, thermogenic heat production, has been a benchmark index of performance in vertebrates. Most measurements of aerobic capacity involve short-term challenges (seconds to hours; e.g. Taylor and Weibel, 1981; Weibel, 1984; Chappell and Snyder, 1984; Garland and Else, 1987; Hammond et al., 2000a) and are often discussed in the context of the absolute expansibility of metabolic power output (i.e. ability to escape predators or survive short-duration cold exposure). Equally interesting from an evolutionary perspective is what is often termed 'sustainable metabolic output,' which is an individual's performance capacity over days or weeks of high-energy demand, such as during lactation, parental care, chronic exposure to cold environments, or migration (e.g. Hammond et al., 1994). Estimates of sustainable metabolic output suggest that birds and mammals expend energy at rates up to 7 times higher than basal metabolism, although 4 times basal is more typical (see references in Peterson et al., 1990; Hammond and Diamond, 1997). Unsurprisingly, there are often significant changes in categories of energy expenditure during different parts of an animal's life history (i.e. during reproduction or dispersal) or during different seasons (summer versus winter). During periods of relative quiescence, endotherms may only expend 2–4 times their basal metabolic rates (BMRs), but they may greatly increase power output when energy demands increase (references cited in Brit-Friesen et al., 1989; Kenagy et al., 1990; Hammond et al., 1994; Koteja, 1996; McDevitt and Speakman, 1994).

Despite extensive research on aerobic performance and sustainable metabolic output in endotherms, little is known about the effect of long-term exposure to harsh or unpredictable environments during development (gestation and post-natal maturation) on either short- or long-term levels of (or limits to) metabolic energy expenditure. It is quite possible that the consequences of exposure to particular environmental conditions may differ depending on whether the exposure was during development or as an acclimatory response during adulthood. In this context a particularly instructive model system for exploring genetic and phenotypic influences on performance is responses to high-altitude environments. For endotherms—especially small ones—high altitudes pose a dual challenge: they are generally colder

than nearby low-altitude habitats, and the availability of oxygen (measured as partial pressure,  $PO_2$ ) is substantially reduced. This puts endotherms at high altitude into a potential double bind: because of cold, they must expend energy at high rates (Hayes, 1989), but because of low atmospheric  $PO_2$  they must do so in hypoxic conditions that may impose limits on aerobic activities such as exercise and thermostatic heat production (e.g. Lenfant, 1973; Snyder, 1981; Chappell et al., 1988).

There have been numerous studies across a wide range of taxa of both genetic adaptation and acclimatory plasticity in response to altitude. However, one species stands out as an especially good model system: the North American deer mouse *Peromyscus maniculatus*. A major emphasis for studies of deer mice has been evolutionary responses to altitude, and one of the best examples of apparent selection on a key physiological trait with relatively simple genetics concerns how deer mouse hemoglobin polymorphisms are adapted to maximize aerobic performance across a wide-altitude range (Snyder, 1978a,b; Chappell and Snyder, 1984; Chappell et al., 1988). The evidence that deer mouse hemoglobins have evolved in response to altitude is threefold: major  $\alpha$ -globins haplotypes (i) have population gene frequencies highly correlated with native altitude; (ii) strongly influence blood oxygen affinity; and (iii) affect short-term maximal aerobic performance of animals (over periods of several minutes). It is important to emphasize, however, that most of Chappell and Snyder's performance data did not incorporate the influence of development and maturation at high altitude. That may be a critical factor because field and lab studies of aerobic performance in deer mice have yielded conflicting results. The maximal aerobic performance of deer mice born and reared in a laboratory colony at 340 m but tested at a high-altitude site (3800 m) after 2 months of acclimation (Chappell et al., 1988) was 62% lower than that of wild deer mice caught at the high-altitude location and tested immediately (Hayes, 1989)—even though many of the laboratory-reared mice were derived from the same high-altitude wild population studied by Hayes. Given this overall genetic similarity, it is important to understand if the performance difference between wild- and laboratory-reared mice was due to acclimation to their local environment

as adults, or was a result of exposure to different environments during development.

Based on the hypothesis that gestational development at high altitudes will influence adult aerobic performance differently than acclimation to high altitudes after gestation, we tested two simple predictions: (1) mice born and tested at low altitudes will have better aerobic performance than those born and tested at high altitudes; and (2) mice born and tested at high altitude will have higher aerobic performance at high altitude than those born at low altitude and tested at high altitude as adults. Finally, we examined the implicit assumption that acclimation is beneficial to performance and predicted that mice born and acclimated at low altitude and tested at high altitude without acclimation (i.e. 'instantaneous' exposure to low  $PO_2$ ) will have reduced aerobic performance compared to mice acclimated and tested at low altitude or mice tested at high altitude after acclimation.

## 2. Methods and materials

### 2.1. Animals and experimental design

We used a total of 45 female and 43 male *Peromyscus maniculatus sonoriensis* between the ages of 50 and 400 days. Mice came from a colony that was 3–6 generations removed from the wild; the initial cohort of 50 animals was trapped on Mt. Barcroft in the White Mountains of eastern California (local altitude 3500–3800 m).

Animals were randomly assigned to in one of four different treatment groups based on the altitude (and hence ambient  $PO_2$ ) of their in utero development and growth (hereafter referred to as the site of birth), and the site where aerobic metabolism was measured at adulthood (hereafter referred to as the site of testing). The groups were: (1) Low Born/Low Tested ( $n=46$ )—mice went through development and growth at low altitude and were tested at low altitude (Riverside, CA; elevation 340 m;  $PO_2=153$  Torr); (2) High Born/High Tested ( $n=14$ )—mice went through development and growth at high altitude and were tested at high altitude (the Barcroft Laboratory of the University of California's White Mountain Research Station; elevation 3800 m; ambient  $PO_2=101$  Torr); (3) Low Born/High Tested ( $n=13$ )—mice went through development and growth at low altitude and were tested at high altitude

after a 3-week acclimation period; (4) Low Born/Instantaneous High Tested ( $n=15$ )—mice that went through development and growth at low altitude and were tested in a hypoxic gas mixture with  $PO_2$  (101 Torr) equivalent to that at Barcroft. The Low Born/Low Tested group was larger than the other groups because much more space was available at our low-altitude lab than at the Barcroft field site.

All mice were housed separately in plastic shoebox cages ( $27\times 21\times 14$  cm<sup>3</sup>) at ambient temperatures between 20 and 24 °C. They were given ad lib food, water and bedding (aspen sawdust). All mice studied at high altitude were moved to the Barcroft Laboratory at 30–35 days of age and tested at 50–70 days of age. The Low Born/Low Tested mice were 60–400 days of age when tested and the Low Born/Instantaneous mice were 100–140 days of age when tested. Preliminary results from an ongoing study of age effects suggest that these age differences probably did not influence aerobic performance.

### 2.2. Measurements

#### 2.2.1. Basal metabolic rate

BMR was measured as oxygen consumption ( $VO_2$ ) during the animals' rest phase (daylight) at approximately 30–31 °C (within the thermal neutral zone (TNZ), Chappell and Holsclaw, 1984), using open-flow respirometry (Withers, 1977; Hammond et al., 2000a). Mice were post-absorptive at the time of measurements. Our system was based on Applied Electrochemistry S-3A/II  $O_2$  analyzers interfaced with Tylan and Applied Materials mass flow controllers and Macintosh computers. To simulate the high-altitude  $PO_2$  of 101 Torr at our low-altitude site, we used a gas mixture of 13.8%  $O_2$  and 86.2%  $N_2$  at the ambient barometric pressure of 730 Torr. At both low- and high-altitude sites, flow rates of dry gas were approximately 600 ml/min corrected to standard temperature and pressure (STPD). We measured  $VO_2$  for 3 h; BMR was estimated as the lowest 10-min continuous average during periods when  $VO_2$  was low and stable.

#### 2.2.2. Maximum aerobic performance ( $VO_{2MAX}$ )

After completing BMR measurements on each individual and allowing a day of rest and ad lib food availability, we used intense treadmill exercise to elicit  $VO_{2MAX}$ , as in previous studies of

deer mice (Chappell and Snyder, 1984). This method uses the same open-circuit respirometry system described for BMR measurements, but with flow rates of approximately 2100 ml/min STPD. As for BMR tests, a 13.8% O<sub>2</sub> gas mixture was used to achieve a high-altitude P<sub>O<sub>2</sub></sub> of 101 Torr at our low-altitude site. We used an enclosed treadmill as the metabolism chamber, and mice were run at increasing speed steps until V<sub>O<sub>2</sub></sub> no longer increased with speed or animals were unable to maintain coordinated running. The air temperature in the treadmill was between 20 and 24 °C. We used a computerized analysis that removed mixing effects that would otherwise distort short-term responses (the ‘instantaneous’ correction; Bartholomew et al., 1981) and determined the highest 1- or 2-min continuously averaged V<sub>O<sub>2</sub></sub>.

### 2.2.3. Other metabolic calculations

From measurements of BMR and V<sub>O<sub>2max</sub></sub> we calculated two other indices of aerobic performance. The ratio of V<sub>O<sub>2max</sub></sub> to BMR (V<sub>O<sub>2max</sub></sub>/BMR; known as aerobic scope; AS) is a measure of the expansibility of metabolic output. Normal AS values for small mammals are between 4 and 13 (Hinds et al., 1993). The difference between V<sub>O<sub>2MAX</sub></sub> and BMR, which we term ‘usable difference’ (UD), is an estimate of the absolute capacity for aerobic power output above maintenance (BMR). These measures are useful estimates of how metabolic energy is partitioned between maintenance and activity, but it is important to recognize that we do not completely understand how metabolic power is utilized during activity. For instance, we do not know if the energy normally used for maintenance (BMR) contributes to the overall activity energy budget (e.g. via transient reductions in maintenance expenditures), or—as assumed in our calculation of UD—that basal energy expenditure is ‘fixed’ and reserved only for maintenance (Ricklefs et al., 1996; Hammond and Diamond, 1997).

### 2.2.4. Hematocrit and hemoglobin

After metabolic measurements were complete, we anaesthetized mice (0.07 ml of sodium pentobarbital injection i.p. (65 mg/ml)) and obtained blood samples (approximately 400 µl in four separate microhematocrit tubes) with heparinized capillary tubes using retro-orbital puncture. For each mouse, two of these samples were centrifuged for 10 min and hematocrit was calculated as the

proportion of packed cells relative to total sample volume. These samples were averaged for the final result. The remaining blood was collected in a small cryo-vial and frozen in liquid nitrogen for subsequent determination of hemoglobin. Total hemoglobin in thawed samples was determined using a Sigma Diagnostic Kit (#525 A). We also tested for a correlation between hemoglobin and hematocrit.

### 2.2.5. Body composition

Because a common response to high altitude is a reduction in body mass, we measured body composition to determine if mass changes were a result of a loss of lean tissue, fat, or both. Body composition data consisted of the following: wet mass, empty dry carcass (mass with organs removed), and dry lean mass. Fat was extracted from the dried carcass using petroleum ether in a Goldfische apparatus (Kerr et al., 1982). We calculated the fat content (percent fat) of each mouse by using the absolute fat mass (carcass mass before extraction – carcass mass after extraction) and then dividing by whole body mass. Lean mass was calculated as whole body mass minus absolute fat mass.

### 2.3. Statistics

Our data consisted of the four independent treatment groups (Low Born/Low Tested, High Born/High Tested, Low Born/High Tested, and Low Born/Instantaneous High Tested) with two levels of sex, several dependent variables (mass, V<sub>O<sub>2MAX</sub></sub>, BMR, hematocrit, body fat content), and two derived dependent variables (AS and UD). All variables were normally distributed (Wilks–Shapiro test of normality; SAS Institute, 1987). We used a 4×2 factor (four treatment groups and two sexes) analysis of variance (ANOVA). In cases where sex was not a significant factor (it was significant for body mass and body composition), we collapsed the data to a 4 factor (treatment group only) ANOVA or ANCOVA (using body mass as the covariate). We tested for interactions between the covariate and the main effect and found none.

We use an alpha of 0.05 for statistical significance. Unless otherwise stated, *F* and *P* values are from these statistical tests; treatment and error degrees of freedom are noted as subscripts to *F* values. The error degrees of freedom vary because

Table 1

Mean ( $\pm 1$  S.E.M. in parentheses) body mass and composition of deer mice in different treatment groups, and  $P$  values for treatment and sex factors

Treatment	Sex	Whole mass (g)	Lean mass (g)	Fat mass (g)
Low Born/Instantaneous High	F	19.04 (1.21)	17.89 (0.89)	1.45 (0.57)
	M	25.68 (1.43)	21.44 (1.06)	4.24 (0.72)
Low Born/Low Tested	F	20.86 (0.73)	17.69 (0.56)	1.97 (0.34)
	M	22.85 (0.70)	20.00 (0.54)	2.64 (0.35)
Low Born/High Tested	F	16.90 (1.21)	15.60 (0.89)	1.30 (0.60)
	M	17.41 (1.13)	16.03 (0.83)	1.38 (0.57)
High Born/High Tested	F	17.36 (1.21)	15.74 (0.89)	1.62 (0.60)
	M	18.76 (1.21)	17.02 (0.89)	1.74 (0.60)
Effects of treatment ( $P$ value)		0.0001	0.0001	0.06
Effects of Sex ( $P$ value)		0.0015	0.0021	0.0233

a few measurements were lost. Because of this we used Type III sums of squares to obtain the  $F$  values. We also tested the correlations between  $VO_{2MAX}$  and BMR, after removing the effects of body mass by using the standardized residuals from a body mass versus metabolic rate (either  $VO_{2MAX}$  or BMR) regression. The significance of those correlations was tested in SAS. In all cases we report the mean  $\pm 1$  S.E.

### 3. Results

#### 3.1. Body mass and composition

There were sex differences in whole fresh body mass, lean body mass, and fat mass, but not for the percent of whole body mass made up of fat (Table 1). Within every treatment group whole body mass, lean mass and fat mass were larger in males than in females (whole mass:  $F_{1,73}=10.9$ ,  $P=0.002$ ; lean mass:  $F_{1,70}=10.2$ ,  $P=0.0021$ ; fat mass:  $F_{1,77}=5.4$ ,  $P=0.023$ ). Gender differences were especially pronounced in the Low Born/Instantaneous High Tested mice.

Lean and whole body masses of mice tested at low altitude (both Low Born/Low Tested and Low Born/Instantaneous High Tested) were 19–24% higher than those of mice tested at high altitudes (lean mass:  $F_{3,74}=9.96$ ,  $P=0.0001$ ; whole mass:  $F_{3,7}=11.9$ ,  $P=0.0001$ ; Fig. 1). There were no significant differences in absolute fat mass (mean =  $2.1 \pm 1.7$  g) or percent fat mass (mean =  $6.9 \pm 10\%$ ) between treatment groups.

Because of sex differences in body mass, we used body mass as a covariate for the remaining

variables, using both whole and lean fresh mass. Both covariates gave comparable results so we report only the former.

#### 3.2. Metabolic data

BMR was not affected by sex but differed between treatment groups, with BMRs of Low Born/Instantaneous High mice averaging approximately 20% higher than those of other groups ( $F_{3,76}=4.2$ ,  $P=0.009$ ; Fig. 2A) after correction for mass differences.

$VO_{2MAX}$  was not affected by sex, but there were significant treatment-group effects ( $F_{3,76}=3.8$ ,  $P=0.014$ ; Fig. 2B). The  $VO_{2MAX}$  of Low Born/Low Tested mice was 11–19% higher than that of high altitude tested mice. Surprisingly, the  $VO_{2MAX}$  of Low Born/Instantaneous High mice was 15% higher than that of Low Born/High Tested mice. Equally surprising was a lack of difference between the  $VO_{2MAX}$ S of Low Born/Low Tested mice and Low Born/Instantaneous High mice.

After accounting for mass differences, Low Born/Low Tested mice had a 20% higher UD than Low Born/High Tested mice ( $F_{3,76}=3.4$ ,  $P=0.0221$ ; Fig. 2C). There were no other differences in UD between treatment groups.

AS ( $VO_{2MAX}/BMR$ ) was significantly different between treatment groups ( $F_{3,76}=3.1$ ,  $P=0.03$ ; Fig. 2D), mainly due to low AS in Low Born/Instantaneous High mice (23% lower than the other three groups).

#### 3.3. Hematocrit and hemoglobin

Mice tested at high altitude (regardless of birth site) had an 8% higher hematocrit and a 10%

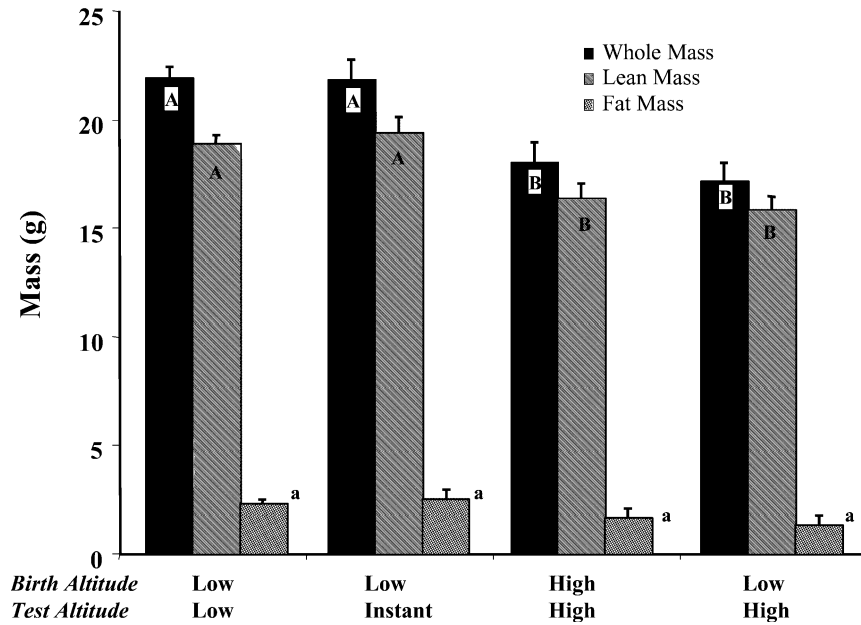


Fig. 1. Body composition in deer mice born at either high or low altitude and tested for metabolic performance at either low or high altitudes. Solid bars represent whole live mass, shaded bars represent lean body mass, and hatched bars represent absolute fat mass. Within a single color bar, letters that are different from each other indicate statistically significant differences. Error bars represent 1 S.E.M.

higher hemoglobin than those tested at low altitude (hematocrit:  $F_{3,82} = 31.8$ ,  $P = 0.0001$ ; hemoglobin:  $F_{3,82} = 5.5$ ,  $P = 0.002$ ; Fig. 3).

### 3.4. Correlations between $VO_{2MAX}$ , BMR, and hematocrit

We found no significant correlations between  $VO_{2MAX}$  and BMR, or between these variables and blood parameters (hemoglobin and hematocrit) for any treatment group (Table 2). There are significant correlations between hematocrit and hemoglobin for mice tested at low altitude (Low Born/Low Tested and Low Born/Instant High groups). When data from all treatment groups were corrected for body mass separately and then pooled in a correlation analysis, we found a marginally significant positive correlation between  $VO_{2MAX}$  and BMR ( $R = 0.222$ ;  $P = 0.047$ ), and significant negative correlations between  $VO_{2MAX}$  and hematocrit ( $R = -0.249$ ;  $P = 0.028$ ) and a positive correlation between hematocrit and hemoglobin ( $R = 0.629$ ;  $P = 0.0001$ ).

## 4. Discussion

The main goal of this project was to understand the effects of sustained exposure to low  $PO_2$  on

aerobic performance, and in particular to distinguish between the effects of developmental versus adult acclimation to low  $PO_2$  (high altitude). Unsurprisingly, we found that deer mice tested at low altitude had better aerobic performance than mice tested at high altitude, regardless of developmental and acclimation history. Paradoxically, we did not find that instantaneous exposure to low  $PO_2$  resulted in reduced aerobic performance at low altitude. Also, although we predicted a better aerobic performance in mice that went through development at high altitudes than those acclimated to that environment as adults, we did not find a clear difference.

## 5. Effects of instantaneous exposure to low $PO_2$

As expected, Low Born/Low Tested mice had a higher aerobic performance than High Born/High Tested mice, presumably because the substantially higher  $PO_2$  at low altitudes (153 vs. 101 Torr in our study) provides a respiratory advantage in oxygen uptake or delivery. Humans and other animals, even high-altitude natives, generally perform better in high  $PO_2$  environments than in low  $PO_2$  environments (Chappell and Snyder, 1984;

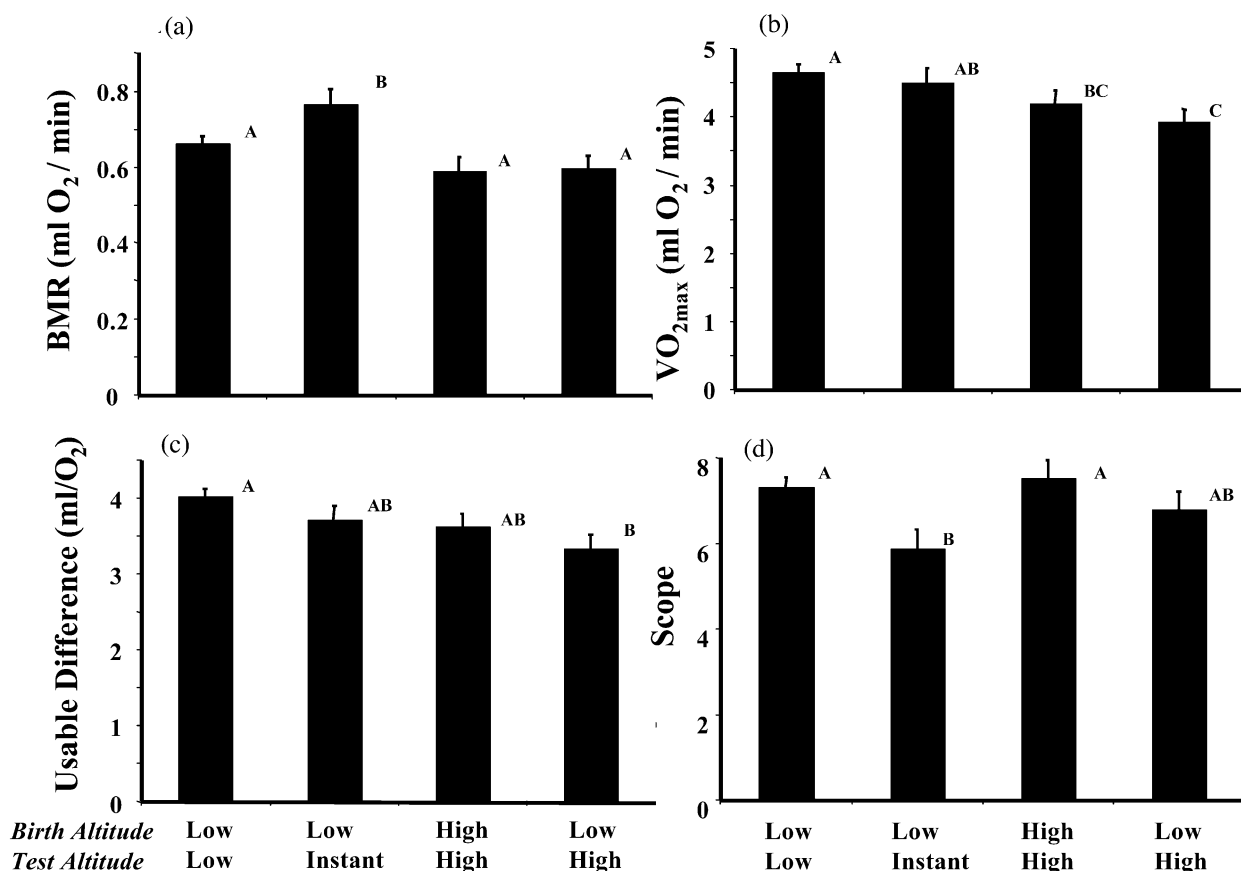


Fig. 2. Metabolic in deer mice born at either high or low altitude and tested at either low or high altitudes for either (A) basal metabolic rates (BMR); (B) aerobic performance ( $VO_{2MAX}$ ); (C) UD between aerobic performance and BMR; (D) AS. All values represent covariate (body mass) adjusted least squares means. Within a histogram, letters that are different from each other indicate statistically significant differences. Error bars represent 1 S.E.M.

Martin and O’Kroy, 1992; Favier et al., 1995b; Widmer et al., 1997; Hochachka, 1998). Therefore, we were puzzled to find that deer mice reared and acclimated to low altitude did not suffer reduced maximal aerobic performance when tested in an ‘instantaneous’ low  $PO_2$  environment. What could be the explanation for this apparent paradox? Many studies on mammals of various sizes and functional capacities (athletic versus sedentary species across several families of mammals) have shown that the diffusive capacity of the lung is not generally a limitation to oxygen transport (cf. Taylor and Weibel, 1981; Weibel et al., 1991; Hsia, 1998). A standard short-term response to low atmospheric  $PO_2$  is a reduction in arterial  $PO_2$  followed by an increase in ventilation (Frappell et al., 1995). An increase in ventilation serves, over the short-term, to increase oxygen uptake and to restore arterial  $PO_2$ , and possibly permit normal levels of short-

term energy expenditure. Presumably, the instantaneous reduction of  $PO_2$  for our animals—equivalent to the  $PO_2$  near the maximum breeding altitude for *P. maniculatus*—would have had to be greater—perhaps considerably greater—to decrease aerobic performance. For example, rats exposed to gas mixtures with lower concentrations of  $O_2$  than we used (10% vs. our 14%) show increases in both ventilation and  $VO_{2MAX}$  relative to normoxia (Mortola et al., 1994). Unfortunately, we did not test mice at a successively lower instantaneous  $PO_2$ , nor were we able to measure their ventilation rate at  $VO_{2MAX}$  to test this hypothesis.

If this scenario is true, why did the mice acclimated to high altitude (either from birth or as adults) have reduced  $VO_{2MAX}$  compared to mice tested at normoxic  $PO_2$ ? Part of the answer may be that the high-altitude treatments were chronic.

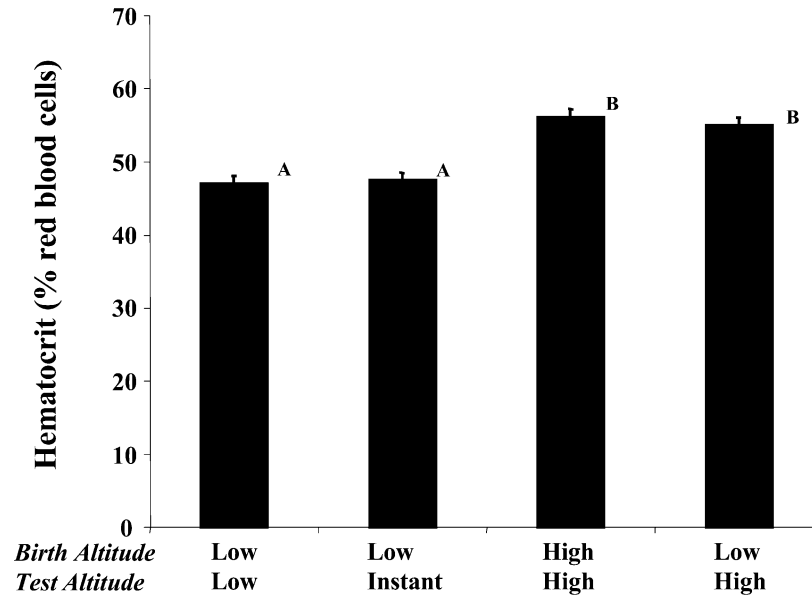


Fig. 3. Hemoglobin (squares) or hematocrit (circles) of deer mice born at either high or low altitude and tested for metabolic performance at either low or high altitudes. Within a line, letters that are different from each other indicate statistically significant differences. Error bars represent  $\pm$  S.E.M.

Short-term responses to hypoxia entail a decrease in arterial  $PO_2$  and increase in ventilation (as mentioned above). As the duration of chronic hypoxia increases, there is a restoration of ventilatory rate with the onset of changes in blood chemistry (either hematocrit or pH). Thus, it may not be reasonable to expect that long- and short-term responses to low  $PO_2$  should be the same.

We also have no clear explanation for why our ‘instantaneous’ exposure deer mice had an elevated BMR. Frappell et al. (1992) found that in 27 species of mammals, including deer mice, a brief hypoxic challenge (10%  $O_2$ , or 40% lower than in our tests) produced a *lower* resting metabolic rate (approximately 35% lower) than in normoxia. There is high variance in the Frappell et al. (1992) data set, but the pattern of reduced metabolism was consistent in nearly all species. Interestingly, Frappell et al.’s deer mice decreased their metabolic rate by only 3% in hypoxia (much less than the average hypoxic decrease in that study)—but it was still lower, rather than higher, than their normoxic metabolic rate. It is possible that our results stem from activity artifacts. Two of our 12 deer mice given instantaneous hypoxia never totally settled in the BMR chamber and their values were 60–90% higher than mice in the rest of the mice in that treatment group. At least one of the

animals was an outlier. When it is removed from the treatment group the difference between the Low Born/Instantaneous High and the Low Born/Low Tested groups disappears.

## 6. Effects of acclimation to high altitude

In general, the many studies of the aerobic physiology of altitude acclimation have shown that performance increases with acclimation time (cf. Favier et al., 1995a; Abdelmalki et al., 1996; Levine and Stray-Gundersen, 1997; Hochachka, 1998), but not necessarily to the level achieved by low-altitude acclimated and tested individuals. The acclimation processes includes phenotypic changes in several physiological and morphological characteristics such as hemoglobin and hematocrit (Hochachka, 1998; Hammond et al., 1999, 2001), muscle fiber types (Kayser et al., 1991; Rosser and Hochachka, 1994), and heart and lung mass (Hammond et al., 1999, 2001). There is also a deterioration of muscle fiber content and reduction of muscle oxidative capacity during severe hypoxia (Abdelmalki, et al., 1996; Hoppeler, 1999). It is apparently only in combination with either increased endurance exercise or cold exposure that muscle oxidative capacity increases with acclimation to high altitudes. Neither of our groups of



Table 2

Correlation coefficients (*R*) and *P*-values for the correlations between the metabolic variables (BMR,  $VO_{2MAX}$  and hematocrit)

Birth altitude Test altitude	Low Low	Low Instant High	High High	Low High	Pooled data
$VO_{2MAX}/BMR$	0.251	0.277	−0.018	−0.048	0.222
<i>P</i>	0.118	0.383	0.953	0.865	0.047
	40	12	14	15	81
$VO_{2MAX}/Hematocrit$	−0.179	0.015	0.083	0.285	−0.249
<i>P</i>	0.275	0.965	0.779	0.323	0.028
	39	11	14	14	78
$VO_{2MAX}/Hemoglobin$	0.068	−0.102	−0.099	0.028	−0.102
<i>P</i>	0.681	0.766	0.736	0.924	0.376
	39	11	14	14	78
BMR/Hematocrit	−0.003	0.333	0.0823	−0.125	−0.118
<i>P</i> =	0.987	0.317	0.778	0.671	0.303
	39	11	14	14	78
BMR/Hemoglobin	0.0388	0.379	0.122	−0.177	−0.020
<i>P</i>	0.815	0.250	0.678	0.546	0.862
	39	11	14	14	78
Hematocrit/Hemoglobin	0.554	0.652	0.502	0.416	0.629
<i>P</i>	0.0001	0.0216	0.0674	0.139	0.0001
	46	12	14	14	86

mice tested at high altitude attained the aerobic performance reported for wild deer mice from the same area (Hayes, 1989), but they were maintained without access to exercise (except for spontaneous activity in the cage), and in a near constant temperature of 22–23 °C so, they presumably did not experience the endurance exercise needed to generate a training effect or the cold exposure needed to induce cold acclimation (Hayes and Chappell, 1986). Both of these factors are important for wild deer mice; in the Barcroft area they frequently move hundreds of meters per night (Hayes, personal communication) and routinely experience freezing temperatures even in summer.

In both groups of high altitude deer mice, we measured a low BMR relative to that of the Low Born/Low Tested mice. Hypometabolism is a common response of many mammals to life at high altitude, in both natives and individuals acclimated to high altitude as adults (cf. Mortola et al., 1994; Frappell et al., 1995; Szewczak, 1998; Mortola, 1999; Singer, 1999). The consequences of the hypometabolic response are often a decrease in core body temperatures and hypothermia (Frappell et al., 1995; Szewczak, 1999). It is not clear whether the decrease in metabolism is a cause of, or a response to lower body temperatures, although there is a clear synergistic effect of both hypoxia

and low ambient temperatures (Szewczak, 1999). We did not measure the TNZ of our sample of deer mice at high altitude and did not measure core body temperatures. However, previous work has shown that the TNZ includes 30 °C at Barcroft (Chappell, 1985) and 30–35 °C at low altitude (Chappell and Holsclaw, 1984). Related research on this species (Szewczak, 1999) has shown that the hypoxic reduction in body temperature is significant, but small when there is not a combined effect of both hypoxia and cold.

## 7. Effects of development in a high-altitude environment

The effects of developmental habitat (both gestational and post-natal) on aerobic performance at altitude have received surprisingly less attention in mammals other than humans. In pregnant mammals in normoxic conditions, the  $PO_2$  of fetal blood may be 17% lower than at sea level (Longo and Pearce, 1998; Singer, 1999). This difference, coupled with a further reduction due to low ambient  $PO_2$  at altitude, could drive acclimatory responses in the fetus. These are known to include hypometabolism, increased ventilation polycythemia, increased organ blood flow, increased hemoglobin, and increased blood oxygen affinity in

newborn mammals (Longo and Pearce, 1998; Richardson and Bocking, 1998; Mortola, 1999; Singer, 1999). Is there evidence that such fetal responses influence adult hypoxia tolerance? There are many studies of aerobic performance and its physiological correlates in humans native to high altitude (cf. Favier et al., 1995a,b; Hochachka, 1998; Beall, 2000), but it is very difficult to control for genetic differences, as well as specific individual historical exposure to other parameters such as cold, exercise, illness, etc. Nonetheless, Favier et al. (1995b) have shown that the aerobic performance of humans native to high altitude increased more than that of low altitude natives when given an acute hypoxic challenge. Thus, high-altitude natives appeared to have a greater expansibility of metabolic response due, according to the authors, to ventilatory, circulatory, and peripheral adaptations during growth. This implicates an effect of developmental plasticity over and above the influence of acclimation during adulthood.

Considerable evidence that suggest the most alveolar development in the mammalian lung occurs after birth (Dunnill, 1962; Weibel, 1967; Cheung et al., 2000), which should offer additional opportunities for post-natal developmental acclimation to altitude. A few studies on laboratory rodents gestated at high altitudes show that developmental exposure to hypoxic environments resulted in larger lung volumes, lung capillary densities, alveolar surface areas and volumes, than normoxic controls (Tenney and Remmers, 1966; Bartlett and Remmers, 1971; Burri and Weibel, 1971; Sekhon and Thurlbeck, 1996). The phenotypic changes in lung volumes result in greater diffusion capacities in hypoxic animals. We have observed significant changes in heart and lung size in very young deer mice born at high altitude (Hammond et al., 2000b), and in adult deer mice born at high altitude (Hammond and Chappell, unpublished data).

Taken together these data are consistent with our initial hypothesis that developmental or early post-natal exposure to high-altitude hypoxia would be an important influence on aerobic performance at low  $PO_2$  in adulthood. However, we found no differences in aerobic performance between High Born/High Tested mice and Low Born/High Tested mice. These somewhat surprising results suggest that the developmental environment may be less important for deer mice that we originally hypothesized. Accordingly, we tentatively con-

clude that the much higher  $VO_{2MAX}$  found in wild mice at our high-altitude site (Hayes, 1989) is due more to exercise conditioning or acclimation to low ambient temperatures (Hayes and Chappell, 1986) than to developmental exposure to low  $PO_2$ .

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