

Deer Mouse Aerobic Performance across Altitudes: Effects of Developmental History and Temperature Acclimation

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Accepted 4/13/2007; Electronically Published 9/6/2007

ABSTRACT

Aerobic physiology at high altitudes has been studied in many animals. Prior work on laboratory-bred deer mice (a species with a wide altitudinal range) showed depression of aerobic capacity at high altitude, even after acclimation. However, wild deer mice show no reduction in thermogenic performance at high altitude, and performance limits seem to be due to physiological and anatomical adjustments to environmental temperature and not to oxygen availability. We asked whether across-altitude performance differences exist in deer mice after accounting for temperature acclimation ($\sim 5^\circ$ and $20^\circ\text{--}25^\circ\text{C}$) and prenatal and neonatal development altitude (340 vs. 3,800 m). We measured maximal thermogenic oxygen consumption ($\dot{V}_{\text{O}_2\text{sum}}$) in cold exposure and ran mice on a treadmill to elicit maximal exercise oxygen consumption ($\dot{V}_{\text{O}_2\text{max}}$). We found a 10% reduction in $\dot{V}_{\text{O}_2\text{max}}$ at 3,800 m compared with that at 340 m; thus, the mice were able to compensate for most of the 37% reduction in oxygen availability at the higher altitude. Development altitude did not affect $\dot{V}_{\text{O}_2\text{max}}$. There was no effect of test altitude or development altitude on $\dot{V}_{\text{O}_2\text{sum}}$ in warm-acclimated animals, but both test and development altitude strongly affected $\dot{V}_{\text{O}_2\text{sum}}$ in cold-acclimated mice, and compensation for hypoxia at 3,800 m was considerably less than that for exercise.

Introduction

The biology of life at high altitudes has long been of interest to ecological and environmental physiologists because these habitats present several clearly definable physiological challenges. Two of the most obvious are low environmental temperatures (typically much cooler than in low-altitude regions at similar latitudes) and reduced oxygen partial pressures (Po_2) due to low atmospheric pressure. For small endotherms living at high altitude, this means that high metabolic rates are necessary to maintain body temperature, and this intense and sustained heat production must be accomplished despite reduced availability of oxygen for aerobic power generation. Low primary productivity at high elevations may add to the challenges of cold and hypoxia by necessitating large home ranges—and hence considerable energetically demanding locomotor activity—to obtain food.

When faced with environmental stressors, animal populations can adjust relevant traits in two ways: by genetic changes in response to selection (i.e., evolution, Darwinian adaptation) or by plastic phenotypic adjustments to conditions experienced within the lifetime of individuals (either reversible [phenotypic flexibility] or irreversible changes; e.g., Rezende et al. 2005). All of these mechanisms can ameliorate performance constraints imposed by high-altitude conditions, but the degree of compensation relative to capabilities at low elevation is variable. Obviously, many aspects of metabolic performance are degraded at extreme altitudes, as is well known from the difficulties experienced by even superbly trained and conditioned human climbers during ascents of very high mountain peaks (e.g., West 2000, 2006; Huey and Eguskitza 2001) and by reductions in human aerobic capacity at high but less extreme elevations (e.g., Cerretelli and Hoppeler 1996; Lindstedt and Conley 2001; Westerterp 2001). On the other hand, animals native to high-altitude regions might be expected to achieve considerable or even complete compensation for low Po_2 through the combined influences of phenotypic plasticity (including effects during development as well as on adults) and evolutionary change. For example, critical levels of Po_2 (lethal limits) for rodents native to high elevations were reported to be lower than for other rodent species, in part due to metabolic suppression in severe hypoxia (Rosenman and Morrison 1975).

One of the best-studied examples of altitude adaptation in small endotherms is the deer mouse *Peromyscus maniculatus* (Wagner), which is native to most of North America and inhabits a very wide elevation range. For example, in a small area

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of eastern California, one subspecies (*P. maniculatus sonoriensis*) occurs at altitudes from below sea level in Death Valley to above 4,000 m in the nearby Sierra Nevada and White mountains. Evolutionary responses to altitude have been clearly demonstrated in the extremely diverse hemoglobin polymorphisms of deer mice. The species' α -globin population gene frequencies are tightly correlated with native altitude (Snyder et al. 1988), strongly influence whole-blood oxygen affinity, and affect maximal whole-animal aerobic performance during exercise and thermogenesis (Chappell and Snyder 1984; Chappell et al. 1988). These studies, which were done with warm-acclimated and mostly lab-born mice reared at low altitude (two or three generations in captivity), found a reduction in maximal exercise and thermogenic aerobic performance at high altitude compared to that attainable at low altitude (regardless of α -globin genotype). Similarly, Hammond et al. (2002) found reduced aerobic capacity in lab-reared deer mice at high altitude (in both cases, the decrease in performance at high altitude was much less than the reduction in Po_2 , indicating considerable compensation). However, tests of wild-caught deer mice across a range of altitudes (~300 to ~3,800 m) revealed strong effects of seasonal temperature variation but no effect of altitude on either average daily energy expenditures or maximal thermogenic metabolism (Hayes 1989a, 1989b). Taken together, these findings suggest that phenotypic flexibility or plasticity—perhaps contingent on development at high altitude—can completely compensate for high-elevation hypoxia, at least for elevations up to 3,800 m. Consistent with that conclusion, several organ morphological and blood parameters in deer mice (Hammond et al. 1999, 2001) show considerable plasticity (both developmental changes and adult acclimation) in response to elevation gradients. It should be noted that the deer mice in these studies were derived from a population native to high altitude; it is unclear whether the same plasticity is present in other *P. m. sonoriensis* populations from low altitude or in other subspecies of deer mice without an evolutionary history at high altitudes.

Here we use deer mice to test two related questions about maximal aerobic performance at high altitude: first, whether exposure to high-altitude conditions during early ontogeny (gestation, birth, and early postnatal development) is mandatory to achieve maximal acclimation and, second, whether acclimation to 3,800 m—possibly including in situ gestation and early postnatal development at that altitude—can fully compensate for high-altitude hypoxia and enable mice to achieve equal aerobic performance at low and high elevations. Unlike much of the previous work on these topics, we tested our deer mice during intense forced exercise as well as acute cold exposure, both of which are likely to be ecologically relevant for this species (Hayes and O'Connor 1999). Also, we used animals that were acclimated to both warm (20°–24°C) and cold (~5°C) environmental temperatures. Inclusion of cold is important because cold-acclimated deer mice recruit an additional effector

organ (brown adipose tissue [BAT]) into their thermogenic response to supplement skeletal muscle shivering (e.g., Heldmaier and Buchberger 1985; Himms-Hagen 1990) and can attain much higher rates of oxygen consumption than are possible for warm-acclimated animals (e.g., Hayes and Chappell 1986; Chappell and Hammond 2004; Rezende et al. 2004). Moreover, wild deer mice are naturally cold acclimated in winter at low altitudes and throughout the year at high altitudes: even in summer, nocturnal temperatures at our high-altitude study site are often below freezing and rarely exceed 10°C. For maximum consistency in across-altitude comparisons, we used mice with similar genetic backgrounds (wild mice or lab-bred animals derived from the same wild population) and the same equipment for all measurements.

Material and Methods

Animals

We studied a western subspecies of deer mice (*Peromyscus maniculatus sonoriensis* Le Conte) derived from a population native to the vicinity of our field site at the University of California's Barcroft Laboratory in the White Mountains of eastern California (local elevation 3,500–4,000 m). The captive-bred mice were fifth- to eighth-generation descendants from 38 wild individuals collected near Barcroft; therefore, the genetic backgrounds of captive-bred and wild-caught mice were quite similar. The captive colony was maintained at the University of California, Riverside (elevation 340 m; ambient $\text{Po}_2 = 153$ –155 torr, depending on weather effects on barometric pressure). Breeding was managed to maximize outcrossing, and there was no intentional selection, except that the founding population was tested to insure that none carried Sin Nombre virus (SNV), a North American form of hantavirus (O'Connor et al. 1997).

Some animals ($N = 127$) were born, acclimated, and tested at Riverside; we refer to these as low-born, low-tested (LBLT) mice. Some ($N = 113$) were kept at room temperature (20°–24°C) before measurements; others ($N = 14$) were cold-acclimated for 6–8 wk in an environmental room maintained at 5°C. In June or July (different years), 57 known-age Riverside-born mice were brought to the Barcroft Laboratory (elevation 3,800 m; ambient $\text{Po}_2 = 100$ –102 torr) for acclimation to altitude (at least 40 d) and testing; these are abbreviated as low-born, high-tested (LBHT) mice. All LBHT mice were born the previous winter or spring and were less than 7 mo old. Seven pairs of LB mice were allowed to breed at Barcroft, and we refer to the resulting 35 offspring as high-born, high-tested (HBHT) mice. Most of the mice kept at Barcroft were housed in a mouse-proof room maintained at 20°–24°C. The Barcroft Laboratory lacks a temperature-controlled environmental room, so 15 mice (13 LB, two HB) were cold acclimated for >5 wk by keeping their cages in a mouse-proof outside shelter exposed to local temperature. Outside temperatures typically

fell below 10°C during the animals' nocturnal activity periods (Fig. 1); temperatures in the mouse-proof enclosure averaged $6.0^\circ \pm 4.2^\circ\text{C}$ at night and $10.9^\circ \pm 4.8^\circ\text{C}$ during the day, with a mean of 8.4°C (readings made every 4 min in July, August, and early September 2006; Hobo Pro datalogger, Onset Computer, Bourne, MA). All Barcroft mice experienced ambient photoperiods (roughly 13L:11D at the dates of measurements). At both altitudes, deer mice were housed in standard plastic small-mammal cages (27.5 cm L \times 17 cm W \times 12 cm H) provided with bedding (wood shavings and cotton) and ad lib. water and rodent chow. Breeding adults were housed in pairs; offspring used in experiments were assigned identifications at weaning and housed individually for the remainder of the study.

Logistical constraints prevented us from producing sufficiently large numbers of captive-bred, cold-acclimated HBHT mice. Therefore, 42 wild deer mice (abbreviated as W mice) were captured near the Barcroft facility in folding aluminum live traps (Sherman, Tallahassee, FL) baited with peanut butter. Traps were opened at dusk (roughly 1900 hours local time) and checked at 2200 hours, midnight, and dawn (about 0630 hours). Captured mice appeared normal, with no behavioral indications of hypothermia. Thermogenic performance was tested within 7 h after the time of capture, and mice were released after measurements were completed. The W mice were marked at the base of the tail with a waterproof pen in order to identify recaptures (individuals were tested once, and recaptures were released immediately). Although very long-distance immigration from low altitudes (\sim 350–500 m) in the adjacent Fish Lake and Owens Valleys—a lateral distance of many kilometers—cannot be completely ruled out, we assume that W mice were gestated and born and had developed at high altitude (i.e., HBHT) and were cold acclimatized from prolonged exposure to low nocturnal temperatures (Fig. 1).

All animal procedures were approved by the UC Riverside Institutional Animal Care and Use Committee (protocol A-M 0408026) and conform to U.S. National Institutes of Health Guidelines (NIH publication 78–23) and U.S. laws. Wild mice were captured under the auspices of California Department of Fish and Game collecting permits.

Gas Exchange Measurements

We used positive-pressure, flow-through respirometry to determine rates of oxygen consumption (\dot{V}_{O_2} ; O_2 concentration measured using an Ametek/Applied Electrochemistry S-3A oxygen analyzer, Pittsburgh, PA) and, for some tests, carbon dioxide production (\dot{V}_{CO_2} ; CO_2 concentration measured using a Sable Systems CA-2A CO_2 analyzer, Henderson, NV). We regulated airflow \pm 1% with an upstream mass flow controller (Tylan, Bedford, MA). About 100 mL min^{-1} of excurrent gas was subsampled and analyzed for O_2 and CO_2 . At both Riverside and Barcroft, the mass flow controller was periodically cali-

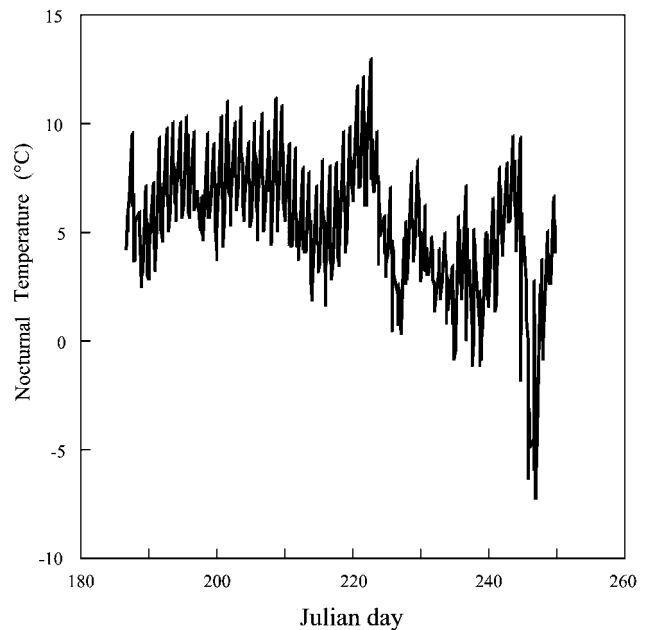


Figure 1. Ambient temperatures at the Barcroft Laboratory in the White Mountains (3,800 m) in the 9 wk preceding $\dot{V}_{\text{O}_2\text{sum}}$ measurements on wild deer mice (July, August, and September 2004). Data are air temperatures measured at a height of about 1.5 m above ground level between dusk and dawn (the activity period of deer mice). At night, the near-ground temperatures experienced by small mammals are often colder than 1.5-m air temperatures, but small-scale microclimate differences may be present and can be exploited by mice to reduce cold exposure.

brated for each gas used (air or heliox; 21% O_2 , balance helium) with a dry volume meter. The S-3A was calibrated against atmospheric air (20.95% O_2) and pure nitrogen, and the CA-2A was calibrated with CO_2 -free air (scrubbed with soda lime) and a certified gas containing a known fraction of CO_2 (0.261%). Data from gas analyzers and other instruments were recorded on Apple Macintosh computers equipped with A-D converters from Sable Systems or National Instruments (Austin, TX) and “Labhelper” software (Warthog Systems, <http://warthog.ucr.edu>).

We dried subsampled gas with magnesium perchlorate and calculated \dot{V}_{CO_2} (mL min^{-1}) as

$$\dot{V}_{\text{CO}_2} = \dot{V} \frac{[(F_e\text{CO}_2 - F_i\text{CO}_2) - F_e\text{CO}_2 \times (F_i\text{O}_2 - F_e\text{O}_2)]}{1 - F_e\text{CO}_2}, \quad (1)$$

where \dot{V} is flow rate ($\text{mL min}^{-1} \text{ STPD}$) and $F_i\text{CO}_2$ and $F_e\text{CO}_2$ are the fractional CO_2 concentrations of incurrent and excurrent gas, respectively ($F_i\text{CO}_2$ was 0.00035–0.0004). Similarly, $F_i\text{O}_2$ and $F_e\text{O}_2$ are the fractional incurrent and excurrent O_2 con-

Table 1: Experimental groups of deer mice, showing numbers of individuals, mean values \pm SD for mass, maximal oxygen consumption in exercise ($\dot{V}_{O_2\max}$; mL O₂ min⁻¹), and maximal oxygen consumption in thermogenesis ($\dot{V}_{O_2\text{sum}}$; mL O₂ min⁻¹)

Birth Altitude, Test Altitude, and Acclimation Temperature	$\dot{V}_{O_2\max}$ (N)	$\dot{V}_{O_2\max}$ Mass (g)	$\dot{V}_{O_2\text{sum}}$ (N)	$\dot{V}_{O_2\text{sum}}$ Mass (g)
340 m:				
340 m:				
Warm (~21°C)	4.58 \pm .090 (113)	21.7 \pm 3.02	4.91 \pm .85 (11)	22.6 \pm 4.01
Cold (5°C)	4.81 \pm .095 (14)	23.3 \pm 2.84	8.08 \pm 1.00 (15)	23.2 \pm 2.71
3,800 m:				
Warm (~21°C)	3.61 \pm .62 (26)	17.8 \pm 1.92	4.38 \pm .72 (42)	21.0 \pm 3.33
Cold (~5°C)	4.06 \pm .78 (13)	19.3 \pm 1.97	4.87 \pm 1.75 (13)	19.3 \pm 1.87
3,800 m:				
3,800 m:				
Warm (~21°C)	3.47 \pm .86 (28)	18.8 \pm 5.21	4.32 \pm 1.30 (33)	19.5 \pm 2.91
Cold (~5°C)	4.34 \pm 1.11 (2)	22.3 \pm 1.63	7.35 \pm .23 (2)	23.1 \pm .82
Cold (wild caught)	5.87 \pm 1.03 (42)	18.0 \pm 3.37

Note. Metabolic rates are not adjusted for mass differences among groups.

centrations, respectively (F_iO_2 was 0.2095 and F_eO_2 was always >0.204).

Different conversion equations were used to compute \dot{V}_{O_2} for captive-reared mice and wild-caught mice due to variation in the arrangement of O₂ and CO₂ analyzers. For captive-reared mice, we scrubbed subsampled gas of CO₂ and water vapor (soda lime followed by Drierite) before O₂ analysis. Accordingly, we calculated \dot{V}_{O_2} (mL min⁻¹) as

$$\dot{V}_{O_2} = \dot{V} \frac{F_iO_2 - F_eO_2}{1 - F_eO_2}. \quad (2)$$

Roughly 20% of wild deer mice in the vicinity of the Barcroft Laboratory are antibody positive for SNV (O'Connor et al. 1997). SNV is a dangerous pathogen transmitted in aerosolized urine, by biting, or in feces (U.S. Centers for Disease Control, <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5109a1.htm>). Accordingly, we handled W mice with large rubber-tipped forceps, wore rubber gloves when working with mice or traps, performed all procedures in open areas outside the laboratory building, and sterilized all equipment in contact with animals in 10% bleach. We did not perform exercise tests on W mice because our treadmill could not be nondestructively sterilized after potential SNV exposure. The metabolism chamber and environmental cabinet were set up outside the lab building, with only the tube carrying dry subsampled gas entering the building. This gas was passed through the O₂ sensor, which operates at about 750°C and hence sterilized the gas stream; in this arrangement, the CO₂ sensor was downstream from the

O₂ analyzer. Because CO₂ was not scrubbed before O₂ analysis, we calculated \dot{V}_{O_2} (mL min⁻¹) as

$$\dot{V}_{O_2} = \dot{V} \frac{(F_iO_2 - F_eO_2) - F_eO_2 \times (F_eCO_2 - F_iCO_2)}{1 - F_eO_2}. \quad (3)$$

Tests with a small number of captive-born animals indicated that \dot{V}_{O_2} measured with this system was statistically indistinguishable from that obtained with CO₂-free gas using equation (2). To do this, we dried subsampled excurrent air (Drierite), flowed it through channel 1 of the S-3A and then removed CO₂ with soda lime, dried the gas, and routed it through the second S-3A channel. We recorded both channels, calculated \dot{V}_{O_2} using equation (3) for data from S-3A channel 1 and equation (2) (for CO₂-free gas) for data from S-3A channel 2, determined mean \dot{V}_{O_2} for the entire measurement period (1.6–3.1 h), and used a paired *t*-test to compare \dot{V}_{O_2} for the two methods ($N = 4$, $t = 0.18$, $P = 0.87$; coefficient of variation [CV] = 1.3%).

Maximum Aerobic Capacity in Exercise

The maximum \dot{V}_{O_2} during forced exercise ($\dot{V}_{O_2\max}$) was obtained by running deer mice in an enclosed motorized treadmill respirometer (Chappell 1984; Chappell and Snyder 1984; Hayes and Chappell 1990; Chappell et al. 2003). The treadmill's working section (6 cm W \times 7 cm H \times 13.5 cm L) was supplied with air at 2,100 mL min⁻¹ STP. Mice were placed in the working section, allowed a 1–2-min adjustment period, and then run at increasing speeds, starting at 0.15–0.2 m s⁻¹ and rising in increments of about 0.1 m s⁻¹ every 30–45 s. A test was

Table 2: Effects of body mass (g), birth altitude (340 or 3,800 m), and test altitude (340 or 3,800 m) on maximal oxygen consumption during forced exercise ($\dot{V}O_{2\max}$; mL O_2 min^{-1}) in deer mice

Factor	Mean Square	$F_{2, 194}$	P
Body mass	28.3	78.5	<.0001
Birth altitude	.189	.353	.553
Test altitude	5.26	9.81	.002
Error	.536		

Note. ANCOVA with mass as covariate; overall $r^2 = 0.502$. Acclimation temperature ($\sim 5^\circ$ or $20^\circ\text{--}24^\circ\text{C}$) had no effect in initial analyses and was dropped from the final model; no interaction terms were significant. At low altitude, $\dot{V}O_{2\max} = 0.428 \times \text{mass}^{0.762}$ ($r^2 = 0.365$, $P < 0.001$), and at high altitude, $\dot{V}O_{2\max} = 0.439 \times \text{mass}^{0.727}$ ($r^2 = 0.358$, $P < 0.001$).

terminated when the mouse no longer maintained position on the tread and $\dot{V}O_2$ did not increase with increasing speed; this typically occurred at $0.4\text{--}0.8 \text{ m s}^{-1}$ ($1.5\text{--}2.9 \text{ km h}^{-1}$). All mice showed behavioral signs of exhaustion at the end of exercise (loss of coordination, failure to maintain speed), but they recovered quickly, and none was injured. Reference readings of incurrent air were obtained at the start and end of measurements.

We calculated $\dot{V}O_2$ during exercise using equation (2). Because of the short duration of treadmill tests (most were completed with <10 min of exercise), we applied the “instantaneous” transformation (Bartholomew et al. 1981) to resolve rapid changes in metabolism. Effective volume of the treadmill was 903 mL. We computed $\dot{V}O_{2\max}$ as the highest instantaneous $\dot{V}O_2$ averaged over continuous 1-min intervals, using LabAnalyst.

Maximum Aerobic Capacity in Thermogenesis

Maximum $\dot{V}O_2$ during acute cold exposure (“summit” metabolism; $\dot{V}O_{2\text{sum}}$) was obtained using a highly conductive helium-oxygen gas mixture (heliox; Rosenman and Morrison 1974), as described previously for deer mice (Chappell and Hammond 2004; Rezende et al. 2004). In brief, animals were weighed ± 0.1 g and placed in a 460-mL metabolism chamber supplied with heliox at $1,700 \text{ mL min}^{-1}$ STP. The initial ambient temperature (T_a) in the chamber was 0° to -4°C , and T_a declined several degrees during the measurement period. In heliox, that temperature range generates rates of heat loss greater than the animals’ maximum rates of heat production, which caused body temperature T_b to decline and elicited $\dot{V}O_{2\text{sum}}$. We removed deer mice from the chamber as soon as $\dot{V}O_2$ began to decrease or if $\dot{V}O_2$ did not increase over a $2^\circ\text{--}3^\circ\text{C}$ decline in T_a . Reference readings of incurrent gas were obtained at the start and end of measurements. Immediately after mice were removed, we measured T_b with a 30-gauge thermocouple in-

serted approximately 1.5 cm into the rectum (this was not done with W animals because of the risk of SNV). Because flow rates were quite large relative to the volume of the metabolism chamber, we did not apply the “instantaneous” transformation (Bartholomew et al. 1981). We calculated $\dot{V}O_{2\text{sum}}$ as the highest $\dot{V}O_2$ (computed with eq. [2]) averaged over 1 min, using LabAnalyst software (Warthog Systems, <http://warthog.ucr.edu>).

Statistics

We compared metabolic variables among test groups using ANCOVA with body mass as covariate. Log transformation of mass and $\dot{V}O_2$ slightly increased the significance of mass regressions and ANCOVAs, but there were no qualitative differences from results with untransformed data and P values were always similar. Accordingly, we present analyses of untransformed data. Categorical variables included acclimation temperature (cold or warm), birth altitude (340 or 3,800 m), and test altitude (340 or 3,800 m), depending on the comparison; initial models also included sex. Previous work on this population showed no effect of age within the age range used here, after correcting for mass effects (Chappell et al. 2003). Non-significant interaction terms (i.e., where slopes for $\dot{V}O_2$ vs. mass did not differ among groups) were dropped from final models. Analyses were performed with the GLM procedures in SPSS for Macintosh (SPSS, Chicago). The significance level was 0.05; results are shown as means (or mass-adjusted means) ± 1 SD unless otherwise noted.

Results

Two obese mice (>30 g) were excluded from analyses. Body mass had highly significant effects on $\dot{V}O_{2\max}$ and $\dot{V}O_{2\text{sum}}$ for all tests and all experimental groups (Tables 1–4), even though

Table 3: Effects of body mass (g), birth altitude (340 or 3,800 m), and test altitude (340 or 3,800 m) on maximal thermogenic oxygen consumption ($\dot{V}O_{2\text{sum}}$; mL O_2 min^{-1}) in warm-acclimated deer mice (ANCOVA with mass as covariate)

Factor	Mean Square	$F_{2, 84}$	P
Body mass	5.92	7.10	.00026
Birth altitude	.614	.737	.393
Test altitude	1.45	1.74	.19
Error	.833		

Note. No interaction terms were significant. The overall r^2 of the model was 0.292. In the pooled data, $\dot{V}O_{2\text{sum}} = 0.701 \times \text{mass}^{0.603}$ ($r^2 = 0.298$, $P < 0.001$).

Table 4: Effects of body mass (g), birth altitude (340 or 3,800 m), and test altitude (340 or 3,800 m) on maximal thermogenic oxygen consumption ($\dot{V}O_{2\text{sum}}$; mL O_2 min^{-1}) in cold-acclimated deer mice, including wild (W) mice (ANCOVA with mass as covariate)

Factor	Mean Square	$F_{2,68}$	P
Body mass	15.96	13.0	.00058
Birth altitude	15.47	12.65	.00069
Test altitude	41.0	33.5	<.0001
Error	1.22		

Note. The overall r^2 for the model was 0.511. Acclimation temperature was controlled at 5°C in an environmental room at the low-altitude test site, but at high altitudes, mice were exposed to outside environmental temperature. This averaged 6°C at night (Fig. 1) and 10.9°C during the day, with maximum daytime temperatures of about 25°C. For low-born, low-tested and low-born, high-tested animals ($N = 15$ and $N = 13$, respectively), the relationship between mass and $\dot{V}O_{2\text{sum}}$ was not significant; for high-born, high-tested (HBHT) animals ($N = 44$, HBHT + W), $\dot{V}O_{2\text{sum}} = 0.876 \times \text{mass}^{0.659}$ ($r^2 = 0.543$, $P < 0.001$).

the mass range in the study was not large (about 13.5–29.8 g; mean 20.25 g, CV = 18.8%). In initial tests, sex did not affect $\dot{V}O_{2\text{max}}$ or $\dot{V}O_{2\text{sum}}$ in any combination of birth altitude, test altitude, or acclimation temperature (data not shown) and was not included in subsequent analyses.

We measured $\dot{V}CO_2$ and computed respiratory quotients (RQ; $\dot{V}CO_2/\dot{V}O_2$) for a subset of measurements of $\dot{V}O_{2\text{sum}}$. Unfortunately, equipment constraints precluded $\dot{V}CO_2$ measurements in LBLT warm-acclimated mice. For cold-acclimated mice, birth and development altitude did not affect RQ ($F_{1,115} = 0.247$, $P = 0.62$), but RQ was significantly affected by test altitude ($F_{1,56} = 44.4$, $P < 0.0001$), averaging 0.805 ± 0.025 ($N = 15$) at Riverside and 0.701 ± 0.058 ($N = 44$) at Barcroft. At high altitude, cold-acclimated mice (including W mice) had a lower RQ than warm-acclimated animals (0.701 ± 0.058 , $N = 44$ vs. 0.743 ± 0.050 , $N = 62$; $F_{1,103} = 18.4$, $P = 0.0004$). The W mice had significantly lower RQ than lab-reared HBHT mice (0.696 ± 0.061 vs. 0.740 ± 0.051 ; $F_{1,72} = 10.3$, $P = 0.0019$; $N = 42$ and $N = 31$). These findings must be regarded cautiously because all W mice, but none of the HBHT mice in these comparisons, were cold acclimated and because W mice may have been without food in traps for up to several hours before tests.

Exercise

There were no effects of acclimation temperature on $\dot{V}O_{2\text{max}}$ in an ANCOVA across altitudes (Table 2; $P = 0.66$) or when the analysis was performed separately for the low-

altitude data ($F_{1,126} = 0.10$, $P = 0.75$) and the high-altitude data ($F_{1,63} = 1.16$, $P = 0.28$). Interactions between acclimation temperature and altitude were not significant. The high-altitude comparison must be viewed with caution because the sample size for high-born, cold-acclimated, lab-bred deer mice was quite small ($N = 2$). However, among lab-born HBHT animals, there was little difference between $\dot{V}O_{2\text{max}}$ means for cold-acclimated mice (3.88 ± 0.68 mL O_2 min^{-1}) and warm-acclimated mice (3.55 ± 0.65 mL O_2 min^{-1} ; both at the adjusted mean mass of 18.4 g). Also, another recent study of this population of deer mice found no effect of cold acclimation on exercise $\dot{V}O_{2\text{max}}$ at low altitude (Chappell and Hammond 2004; but see Hayes and Chappell 1986).

For both acclimation temperatures, there was no effect of birth altitude on $\dot{V}O_{2\text{max}}$ among deer mice tested at the high-elevation Barcroft site ($F_{1,63} = 0.55$, $P = 0.51$; Figs. 2A, 3A). When adjusted to the mean mass of 18.4 g, the $\dot{V}O_{2\text{max}}$ of 41

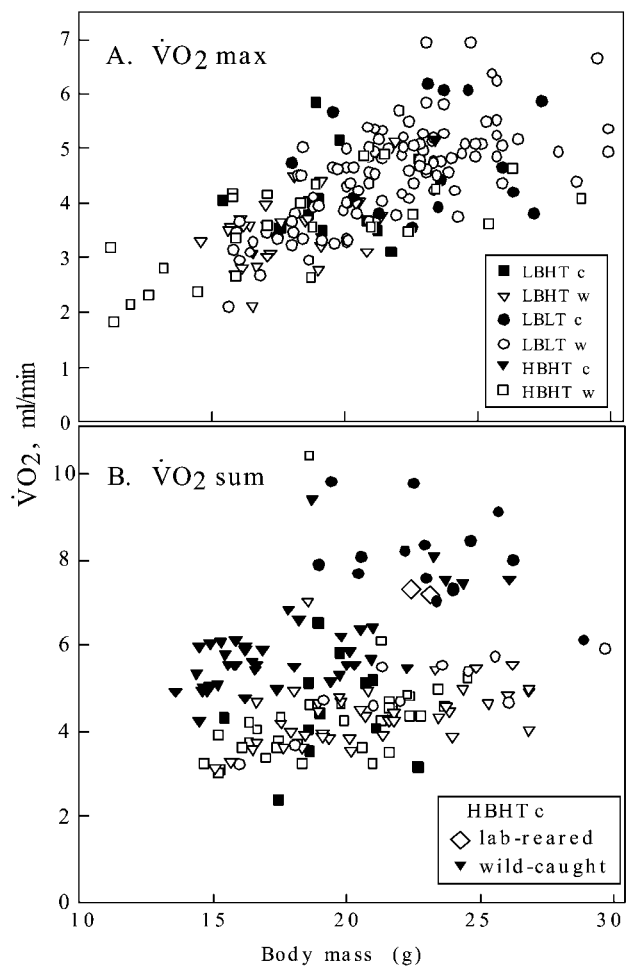


Figure 2. Maximal oxygen consumption of deer mice in forced exercise (A, $\dot{V}O_{2\text{max}}$) and acute cold exposure (B, $\dot{V}O_{2\text{sum}}$).

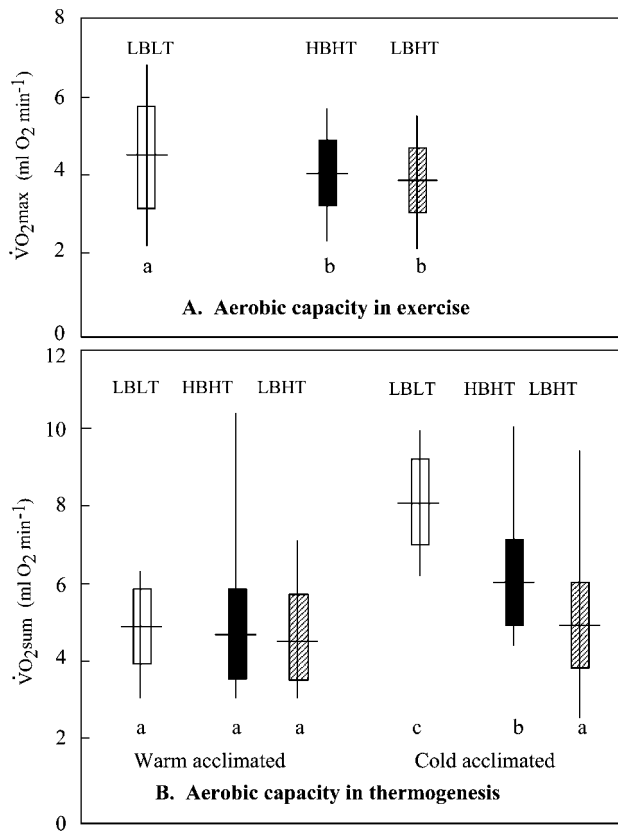


Figure 3. Maximal oxygen consumption of deer mice in forced exercise (A) and during maximal thermogenesis (B; acute cold exposure in heliox) at low and high altitude (340 and 3,800 m, respectively). Vertical lines indicate the range of data, horizontal lines indicate means, and boxes are ± 1 SD. LBLT (open box) = low born, low tested; LBHT (hatched box) = low born, high tested; HBHT (filled box) = high born, high tested (includes wild mice). Groups indicated by different lowercase letters differed significantly from each other. Temperature acclimation had no effect on exercise performance.

LBHT mice was 3.72 ± 0.24 mL O₂ min⁻¹, and the $\dot{V}O_{2\max}$ of 27 HBHT mice was 3.75 ± 0.11 mL O₂ min⁻¹.

The $\dot{V}O_{2\max}$ of mice tested at high altitude (4.01 ± 1.33 mL O₂ min⁻¹, $N = 68$) was 10% less than that of mice tested at low altitude (4.41 ± 1.34 mL O₂ min⁻¹, $N = 131$); both values adjusted to the mean mass of 20.7 g; Tables 1, 2; $P = 0.0007$). The magnitude of this difference in $\dot{V}O_{2\max}$ (Fig. 3A) was smaller than the 37% reduction in P_{O_2} at Barcroft compared to Riverside (calculated as inspired P_{O_2} ; i.e., the P_{O_2} of 100% humidified air at typical deer mouse body temperatures of 37°–38°C).

Cold Exposure

Unlike $\dot{V}O_{2\max}$, the $\dot{V}O_{2\text{sum}}$ during acute cold exposure was strongly affected by the animals' acclimation temperature (Ta-

ble 1; $F_{1,157} = 116$, $P < 0.0001$; see also Hayes and Chappell 1986; Chappell and Hammond 2004; Rezende et al. 2004). For all LBLT, LBHT, HBHT, and W mice combined, the $\dot{V}O_{2\text{sum}}$ of 72 cold-acclimated mice averaged 42% higher than that of 86 warm-acclimated animals (6.31 ± 1.13 vs. 4.33 ± 1.14 mL O₂ min⁻¹, corrected to the adjusted mean mass of 20.1 g).

The $\dot{V}O_{2\text{sum}}$ of warm-acclimated deer mice at Barcroft was unaffected by birth altitude ($F_{1,74} = 0.53$, $P = 0.47$; LBHT $\dot{V}O_{2\text{sum}} = 4.27 \pm 0.96$ mL O₂ min⁻¹, $N = 41$ vs. HBHT $\dot{V}O_{2\text{sum}} = 4.44 \pm 0.97$ mL O₂ min⁻¹, $N = 34$, adjusted to a common mass of 20.3 g). In contrast, birth altitude had a large and significant effect on the $\dot{V}O_{2\text{sum}}$ of cold-acclimated mice tested at Barcroft ($F_{1,54} = 15.3$, $P = 0.0003$; Figs. 2B, 3B), with high-born animals (HBHT and W) having a 28% higher $\dot{V}O_{2\text{sum}}$ than LBHT mice (6.00 ± 1.05 mL O₂ min⁻¹, $N = 44$ vs. 4.69 ± 1.06 mL O₂ min⁻¹, $N = 13$, corrected to the adjusted mean mass of 18.5 g). Moreover, in tests at Barcroft, there was no significant difference between the $\dot{V}O_{2\text{sum}}$ of cold-acclimated LBHT mice and that of warm-acclimated mice (HBHT and LBHT; $F_{2,56} = 0.79$; $P = 0.46$) or between the small number of HBHT lab-reared and cold-acclimated mice and W mice ($F_{1,41} = 1.16$, $P = 0.29$). Nevertheless, we assume that there were differences in acclimation history between W and HBHT mice, so we also compared LBHT and W mice (i.e., with HBHT animals excluded). Results were qualitatively iden-

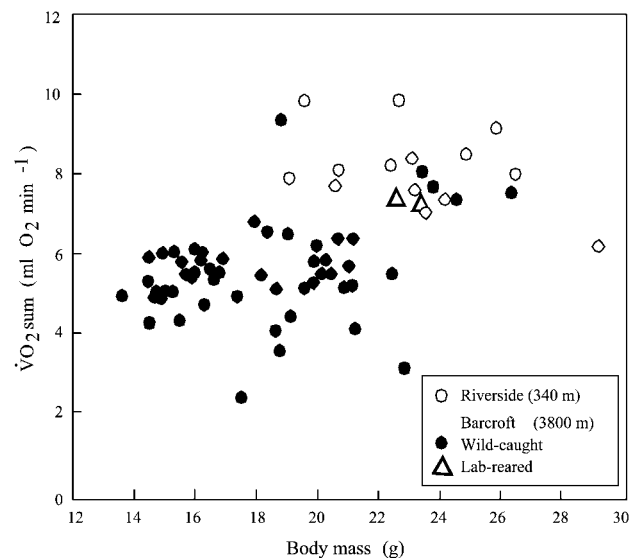


Figure 4. Maximal thermogenic oxygen consumption ($\dot{V}O_{2\text{sum}}$) in deer mice acclimated to low ambient temperatures at low altitude (Riverside, 340 m) and high altitude (Barcroft Laboratory, 3,800 m). Most of the Barcroft mice were wild caught (filled circles) and were acclimated to nocturnal temperatures $< 10^\circ\text{C}$ (Fig. 1); two Barcroft animals (triangles) were lab born but acclimated to outside temperatures; the Riverside-tested animals (open circles) were lab reared and acclimated to 5°C for > 7 wk.

tical to comparisons of LBHT and HBHT + W: birth altitude significantly affected $\dot{V}O_{2\text{sum}}$ ($F_{1,52} = 13.7$, $P = 0.00052$).

To our surprise, we found no significant effects of test altitude on the $\dot{V}O_{2\text{sum}}$ of warm-acclimated deer mice ($F_{1,83} = 0.99$, $P = 0.32$; Fig. 3B), despite the large difference in PO_2 between Riverside and Barcroft. In contrast, cold-acclimated mice had considerably higher $\dot{V}O_{2\text{sum}}$ at low altitude than at high altitude (LBHT, HBHT, and W mice combined; $F_{1,69} = 14.9$, $P = 0.00026$). At Riverside, the $\dot{V}O_{2\text{sum}}$ after cold acclimation was 30% greater than at Barcroft (7.58 ± 1.37 mL O_2 min^{-1} , $N = 15$ vs. 5.84 ± 1.25 mL O_2 min^{-1} , $N = 57$, adjusted to the mean mass of 19.5 g). In a comparison excluding LBHT mice (i.e., LBLT vs. HBHT + W), the difference between Barcroft and Riverside, while still substantial, was smaller (about 21% at the adjusted mass of 19.5 g; 6.16 ± 1.02 and 7.46 ± 1.04 mL O_2 min^{-1} , respectively; $F_{1,56} = 16.1$, $P = 0.00018$). Nevertheless, there was considerable variation, and some Barcroft mice attained $\dot{V}O_{2\text{sum}}$ almost as large as the highest values obtained at Riverside (Fig. 4).

Discussion

Our field site at the Barcroft Laboratory (3,800 m) is high compared to many of the regions inhabited by deer mice, but it is well within the species' altitudinal range: about 500 m lower than the maximum elevations routinely used by free-living *Peromyscus maniculatus sonoriensis*, with correspondingly reduced hypoxic challenges. Ambient PO_2 at Barcroft is about 33% less than that at our UC Riverside laboratory (340 m); accordingly, the difference in inspired PO_2 between the two study sites is approximately 37% (90 vs. 142 torr) after accounting for the humidification of tidal air at typical mammalian body temperatures of 37°–38°C. Without physiological adjustments to compensate for lower PO_2 , this degree of hypoxia at Barcroft would be expected to depress aerobic capacity to well below that attainable at Riverside if oxidative metabolism is constrained by oxygen uptake or delivery (e.g., Lenfant 1973; Hochachka 1998). For example, humans subjected to altitudes of 0, 3,900, 6,200, 7,700, and 9,200 m suffer reductions in $\dot{V}O_{2\text{max}}$ that are directly proportional to reductions in PO_2 across this very large altitude range, suggesting little ability to compensate for reduced PO_2 (Cymerman et al. 1989; Lindstedt and Conley 2001).

Consistent with that prediction, previous work with laboratory-reared deer mice and a few lab-acclimated wild-caught animals revealed declines of roughly 10% in both exercise $\dot{V}O_{2\text{max}}$ and thermogenic $\dot{V}O_{2\text{sum}}$ when the mice were moved from Riverside to Barcroft (e.g., Chappell and Snyder 1984; Chappell et al. 1988). However, those studies were performed after prolonged acclimation to warm temperatures, most of the mice were reared at Riverside, and the test groups consisted of wild-caught population samples and lab-bred lines derived

from numerous wild populations and several subspecies from across a wide geographical range and with considerable variation in native altitude (Snyder et al. 1988). That mixture of contrasting genetic backgrounds and developmental conditions may confound comparisons across altitudes.

The potential importance of normal acclimatization factors—especially birth and development at the test altitude and exposure to typical environmental temperatures T_e —was revealed by studies of $\dot{V}O_{2\text{sum}}$ in freshly captured wild mice tested at their native altitude (Hayes 1989a, 1989b; Hayes and O'Connor 1999). In contrast to results for lab-reared animals, the results for the wild-caught deer mice from the Hayes studies showed that the mice did not seem to suffer reduced $\dot{V}O_{2\text{sum}}$ when tested at high altitude, and differences among high- and low-altitude populations were attributed to variation in T_e , not differences in PO_2 . For example, the $\dot{V}O_{2\text{sum}}$ of Barcroft mice in summer was about the same as that of mice from low altitudes in southern California in winter, presumably because both groups had undergone thermal acclimation to T_e 's of -1° to 4°C . In summer, the $\dot{V}O_{2\text{sum}}$ of Barcroft mice was substantially greater than that of low-altitude mice, when low-altitude nighttime T_e 's for the latter group were $\sim 15^\circ$ – 20°C .

A recent study (Hammond et al. 2002), using lab-reared, warm-acclimated deer mice derived from wild Barcroft ancestors, found an 11%–19% decline in exercise performance ($\dot{V}O_{2\text{max}}$) at Barcroft compared to low altitude (Riverside). Those results are consistent with the early data of Chappell and Snyder (1984) but at odds with Hayes's results for $\dot{V}O_{2\text{sum}}$. Additionally, Hammond et al. found no difference in $\dot{V}O_{2\text{max}}$ at Barcroft between lab-raised animals born at high altitude and those acclimated to high altitude as adults. The latter finding suggests that in situ gestation and development are not necessary to achieve maximal exercise performance at high altitude.

One puzzling aspect of much of the previous work concerns the magnitude of $\dot{V}O_{2\text{sum}}$ following cold acclimation. For wild-caught mice, Hayes (1989a, 1989b; Hayes and O'Connor 1999) reported a $\dot{V}O_{2\text{sum}}$ of roughly 0.25 mL g^{-1} min^{-1} for cold-acclimatized animals at both low and high altitude (the $\dot{V}O_{2\text{sum}}$ of wild-caught low-altitude mice acclimated to 14° – 25°C in May was about 0.18 mL g^{-1} min^{-1} ; Hayes 1989a). However, the $\dot{V}O_{2\text{sum}}$ following laboratory cold acclimation at low altitude is considerably higher, sometimes exceeding 0.35 mL g^{-1} min^{-1} (this study; Chappell and Hammond 2004; Rezende et al. 2004). A possible explanation for the difference is measurement technique: to elicit $\dot{V}O_{2\text{sum}}$, Hayes used a refrigerated closed-circuit wind tunnel flushed with air (as did Chappell and Snyder [1984] for their article), while the other studies relied on standard metabolism chambers using a heliox atmosphere to enhance heat loss. It is conceivable that the extremely high convective heat loss in a cold wind tunnel may depress T_b so rapidly that animals do not have time to achieve maximal $\dot{V}O_2$ before thermogenesis is suppressed by hypother-

mia. Whatever the reason for the difference, these findings suggest that a heliox-elicited $\dot{V}O_{2\text{sum}}$ for cold-acclimated mice at high altitude might be greater than expected from Hayes's wind tunnel data. If so, it is unclear which method is the more useful approximation of conditions experienced by wild deer mice.

In this study, we used deer mice of common genetic origins (the lab animals and wild-caught mice were derived from the same Barcroft wild population) and, as much as possible, controlled for birth and development altitude, test altitude, and acclimation temperature. Our first major result is partially consistent with Hayes's suggestion that with adequate acclimation and in certain testing protocols, deer mice at Barcroft do not suffer reduced aerobic capacity compared to mice tested at low altitude: we observed no effect of test altitude on the heliox-elicited $\dot{V}O_{2\text{sum}}$ of mice acclimated to warm temperatures. Although exercise $\dot{V}O_{2\text{max}}$ was slightly reduced at high altitude, consistent with earlier studies (Chappell and Snyder 1984; Chappell et al. 1988), the reduction in $\dot{V}O_{2\text{max}}$ was much less than the reduction in Po_2 at Barcroft compared to Riverside. Thus, deer mice can largely or completely compensate for low Po_2 at our 3,800-m test site. At either altitude, the $\dot{V}O_{2\text{sum}}$ of cold-acclimated mice was considerably greater than the maximal $\dot{V}O_2$ in any other performance test.

Our interpretation of these findings is that the uptake and transport of oxygen by central organ systems (lungs, heart, and circulation) is not limited by low inspired Po_2 at Barcroft during thermogenesis in warm-acclimated mice and is only moderately reduced during exercise, but hypoxia becomes strongly limiting for the very high rates of oxygen flux required to support maximal thermogenesis following cold acclimation. This does not rule out the possibility that mice acclimated to Barcroft underwent substantial physiological or morphological changes, such as increased heart and lung size (Hammond et al. 2001), even though there were few aerobic capacity differences across altitudes in warm-acclimated animals. Speculatively, exercise $\dot{V}O_{2\text{max}}$ in all mice and $\dot{V}O_{2\text{sum}}$ in warm-acclimated mice may be constrained less by upstream stages in the oxygen transport pathway (lungs, heart, circulatory system) than by the downstream transfer of oxygen from blood to aerobic effector tissues (primarily skeletal muscle) or possibly by factors other than oxygen delivery per se. We also speculate that following cold acclimation and the concomitant hyperplasia of BAT (e.g., Heldmaier and Buchberger 1985; Himms-Hagen 1990), the much greater combined demand for oxygen by two effector tissues (both muscle and BAT) does encounter a strong upstream constraint on oxygen flux and that mice reach their maximal capacity for oxygen transport.

Our second major finding is that birth site (i.e., the altitude of gestation, birth, and early postnatal development) has no effect on maximal aerobic performance at high altitude, except for the most demanding conditions of metabolic power output: in cold-acclimated animals during

maximal thermogenesis. After cold acclimation, high-born mice had greater thermogenic performance at Barcroft than low-born mice acclimated to high altitude and low T_a as adults (23% greater $\dot{V}O_{2\text{sum}}$ in HBHT mice than in LBHT mice; Fig. 3B). That statement requires a caveat: the majority of our cold-acclimated HBHT mice were wild caught and hence may have been affected by factors not pertinent to captive-reared HBHT animals. The most obvious difference is exercise conditioning in W mice, which need to travel over long distances to obtain food, find shelter, and so on, and hence they are likely to be more active than lab-housed mice. However, exercise and heat production appear to be largely separate functions in cold-acclimated deer mice (Chappell and Hammond 2004). Another likely difference is the consistency of the thermal regime. Deer mice in laboratory cold-acclimation experiments are continually exposed to low temperatures, while wild mice at high altitude presumably spend much of their time in insulated nests that may approximate thermoneutral conditions. In our experimental outdoor enclosure at high altitude, nocturnal temperatures averaged 6°C, and diurnal temperatures averaged about 11°C. Additionally, wild mice probably use microhabitat selection and other behavioral mechanisms to reduce cold exposure. Hence, if cumulative daily cold exposure and average daily temperature are important determinants of the cold acclimation response, then for a given low-temperature condition, one might expect greater thermogenic capacity after laboratory acclimation. Nevertheless, it is worth noting that the mean $\dot{V}O_{2\text{sum}}$ for our small sample of captive HBHT mice was indistinguishable from that for W mice (6.40 vs. 5.85 mL O_2 min^{-1} , at the adjusted mean mass = 17.9 g; $F_{1,43} = 1.16$, $P = 0.29$; Fig. 4) and that like wild mice, the captive HBHT mice were exposed to fluctuating outdoor temperatures during their acclimation period.

Given the substantial elevation of aerobic capacity following cold acclimation (increases of 50% or more have been reported in deer mice; Rezende et al. 2004), it is curious that there was no difference between the $\dot{V}O_{2\text{sum}}$ of low-born, cold-acclimated mice at high altitude and the $\dot{V}O_{2\text{sum}}$ of warm-acclimated mice at low altitude (Fig. 3). Because cold acclimation recruits BAT as a primary thermogenic effector in addition to shivering in skeletal muscle, the similarity between these groups is probably coincidental: the LBHT cold-acclimated animals had the benefit of increased BAT but the constraint of hypoxia and vice versa for the LBLT warm-acclimated mice.

In summary, deer mice acclimated to 3,800 m can largely or completely compensate for high-altitude hypoxia during exercise and, for warm-acclimated animals, during thermogenesis. During exercise, mice acclimated and tested at 3,800 m suffer a ca. 10% deficit in aerobic performance compared to low-altitude aerobic capacity, despite a 37% reduction in oxygen availability: a compensation of more than 70%. In warm-acclimated animals tested in thermogenesis at 3,800 m, compensation is 100%. Moreover, lack of any effect of birth altitude

on these traits shows that development at high altitude is not necessary to achieve maximal exercise or warm-acclimated thermogenic performance there. Only under conditions requiring the greatest oxygen flux—intense thermogenesis after cold acclimation—are the effects of altitude hypoxia pronounced, with development at altitude required to achieve the highest attainable performance, and relatively little compensation for reduced Po_2 .

Acknowledgments

Jack Hayes provided the initial cohort of deer mice to establish our laboratory colony. The staff at the Barcroft Laboratory of the White Mountain Research Station provided indispensable help and hospitality. The work was supported in part by University of California, Riverside (UCR), intramural research awards and in part by National Science Foundation grant IBN-0111604 (K. A. Hammond and M. A. Chappell). We thank E. Hice and J. Urrutia in the UCR Biology machine shop for constructing the metabolism chambers, environmental cabinet, and treadmill. Two anonymous reviewers provided numerous helpful comments on an initial version of the article.

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