Aerobic capacity and running performance across a 1.6 km altitude difference in two sciurid rodents

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SUMMARY

Hypoxia at high altitudes is often assumed to constrain exercise capacity, but there have been few high- *versus* low-altitude comparisons of species native to a wide range of altitudes. Such studies are ecologically realistic, as wild-caught animals tested at their native altitude are presumably maximally acclimated (*via* phenotypic plasticity) or adapted (by evolutionary change) to that altitude. We compared aerobic performance, measured as maximum oxygen consumption in forced exercise ($\dot{V}_{O_2,max}$), and voluntary wheel-running in two species of sciurid rodents captured and tested at field sites that differed in altitude by 1.6 km (2165 m *versus* 3800 m). We found reduced $\dot{V}_{O_2,max}$ at 3800 m in least chipmunks (*Tamias minimus*) but no significant effect of altitude on $\dot{V}_{O_2,max}$ in golden-mantled ground squirrels (*Spermophilus lateralis*). Individuals of both species averaged several km day^{-1} in wheels. Most behavioral indices of voluntary running (including mean and maximum speeds, time spent running, daily running distance, and the number and duration of running bouts) were unaffected by altitude, even in the species with reduced $\dot{V}_{O_2,max}$ at high altitude. Metabolic rates during running and energy costs of transport differed to some extent across altitudes but in different ways in the two species. At both test sites, voluntary running by both species was almost exclusively at speeds well within aerobic limits. We conclude that substantial differences in altitude do not necessarily result in differences in aerobic capacity in small mammals and, even if $\dot{V}_{O_2,max}$ is reduced at high altitude, there may be no effect on voluntary running behavior.

Key words: aerobic capacity, altitude, hypoxia, locomotion, small mammal.

INTRODUCTION

The effects of high-altitude hypoxia on vertebrate aerobic performance have been studied by generations of regulatory and evolutionary physiologists. This continuing interest stems from the critical importance of aerobic metabolism, the simple and non-redundant 'pathway for oxygen' from the atmosphere through the respiratory and cardiovascular systems to the mitochondria (Weibel, 1984), and the tight stoichiometry between oxygen uptake and aerobic power output. Moreover, the key environmental parameter that varies with altitude (oxygen availability) and several associated whole-animal performance traits [maximal oxygen consumption ($\dot{V}_{O_2,max}$), exercise capacity, etc.] can be conveniently and accurately measured. It is often assumed that living at high altitude reduces aerobic performance, and, indeed, some detrimental effects of altitude are obvious. For example, it is both intuitive and well documented that exercise capacity is degraded at extreme altitudes, e.g. above ~5500 m, as shown by the difficulty experienced by even the most elite human mountaineers when climbing high peaks (West, 2006; Huey et al., 2007). However, numerous species of vertebrates live permanently or seasonally at altitudes from 3500 to 5500 m and, despite considerable study, how – or if – these altitudes affect performance remains an open question. Theoretically, if oxygen availability and delivery are directly limiting to aerobic power production, one would expect aerobic performance to scale approximately in proportion to oxygen availability (i.e. to atmospheric or inspired P_{O_2}). An alternative view is that high-altitude natives are likely to have physiological, morphological or biochemical adjustments – mediated by mechanisms of phenotypic plasticity or flexibility, or *via* genetic adaptation (evolutionary change) – that partially or completely compensate for hypoxia.

Examples of both responses to hypoxia can be found in the literature. In humans acclimated to different altitudes – even lifelong residents – the upper limit to aerobic performance is highest at sea level and declines in approximate proportion to the fall in P_{O_2} as altitude increases (e.g. West et al., 1983; Cymerman et al., 1989; Lindstedt and Conley, 2001). By contrast, laboratory rats (*Rattus norvegicus*) acclimated to a barometric pressure of 380 torr (equivalent to about 5500 m; 1 torr≈133 Pa) show a reduction in exercise aerobic capacity of only 34% compared to rats acclimated to 740 torr (Gonzalez et al., 1993), even though inspired P_{O_2} at 380 torr is less than half that at 740 torr. Similarly, laboratory-reared deer mice (*Peromyscus maniculatus*) native to and acclimated to 3800 m (approximately 471 torr) show a reduction in exercise aerobic capacity of only 10% compared with their low-elevation performance, despite a 37% difference in inspired P_{O_2} (Chappell et al., 2007a). In a study of wild-caught deer mice tested *in situ* across a 3500 m altitude range in California, USA, Hayes found that aerobic capacity in thermogenesis was affected by seasonal temperature differences but not by altitude (Hayes, 1989a; Hayes, 1989b). The findings for rodents indicate considerable compensation for altitude hypoxia. However, other work with cold-acclimated, cold-exposed deer mice found that maximal oxygen uptake in thermogenesis is sharply reduced at high altitude, and compensation for hypoxia is substantially less than during exercise (Chappell et al., 2007a).

Aside from humans, laboratory rats and deer mice, the effects of altitude on vertebrate aerobic capacity are surprisingly little-studied. Few investigators have performed across-altitude comparisons of freshly captured wild individuals tested at their native altitudes [but see Hayes (Hayes, 1989a; Hayes, 1989b)]. Although not without

interpretive problems, such tests are important because free-living wild animals – unlike animals housed in typical laboratory environments – are exposed to numerous factors besides P_{O_2} that could influence aerobic capacity, including variable ambient temperature, limited food and the need for extensive locomotor behavior. Moreover, it is reasonable to expect that wild-caught animals are fully acclimated to local conditions *via* phenotypic plasticity and flexibility (and perhaps genetic adaptation) and hence should yield the most ecologically realistic measures of aerobic physiology. It is also worth noting that many studies of altitude physiology focus on the upper limits to performance, i.e. brief episodes of very intense exercise or heat production. Upper limits are interesting for many reasons but submaximal 'routine' activities, such as foraging, territorial patrolling, etc., may be as important for fitness as peak power output, and these might also be influenced by altitude hypoxia. We are aware of no studies of wild-caught species that examined how voluntary aerobic power use and locomotor behavior vary with altitude.

To explore the potential effects of altitude on maximal and routine aerobic performance in wild species, we studied two sciurid rodents native to a broad range of elevations in western USA. We worked at two field stations that differed in altitude by 1.6 km and tested freshly captured individuals at each site. Oxygen availability (P_{O_2} in inspired air) differed by 26% at the two locations, and we tested several simple hypotheses based on the assumption that reduced P_{O_2} at high altitude would suppress aerobic metabolism. First, we expected that the upper limit of aerobic power production (maximum oxygen consumption) in forced exercise would be lower at the high elevation site. Second, we expected that voluntary exercise performance (distance run, speeds attained, duration of running bouts, energy used during running) would also be reduced at high altitude. Finally, we tested whether the choice of running speeds and power outputs would vary with altitude, since speed affects both rates of oxygen use and the efficiency of transport (e.g. Taylor et al., 1970; Taylor et al., 1982).

MATERIALS AND METHODS
Animals
We studied wild-caught golden-mantled ground squirrels (*Spermophilus lateralis trepidus* Taylor) and least chipmunks (*Tamias minimus scrutator* Hall and Hatfield) in Mono County, eastern California, USA. The lower-altitude site was the vicinity of the University of California's Sierra Nevada Aquatic Research Laboratory (SNARL) on Convict Creek on the eastern slope of the Sierra Nevada (37°36.49′N, 118°49.35′W; local elevation 2150–2200 m; laboratory at 2165 m). The high-altitude site was the University of California's Barcroft Laboratory in the White Mountains (37°35.00′N, 118°14.13′W; local elevation 3560–4000 m; laboratory at 3800 m). The linear distance between SNARL and Barcroft is approximately 53.5 km.

Field work took place in July and August 2005, 2006 and 2007 at SNARL, and in August and September 2008 at Barcroft. At both sites, study periods were timed to occur after young became independent of their mothers. Animals were captured in aluminum live-traps (Sherman XLKR, Tallahassee, FL, USA). Traps were sheltered from sunlight and wind and baited with rolled oats, birdseed, raisins and peanut butter. We opened traps after dawn and closed them before sunset. In hot weather (mainly at SNARL), traps were checked approximately hourly and closed in the middle of the day. Captured animals were immediately transported to the lab for measurements (see below). After tests were completed, animals were released unharmed at the site of capture (usually on the day of capture; individuals tested for voluntary behavior were released the following day).

Animals were trapped under the auspices of California Department of Fish and Game scientific collecting permits. All procedures were approved by the University of California, Riverside and University of California, Santa Barbara Institutional Animal Care and Use Committees and conform to US National Institutes of Health Guidelines (NIH publication 78–23) and US laws.

Respirometry
We measured metabolic rates as oxygen consumption ($\dot{V}_{O_2}$) using open-circuit respirometry. Outside air was supplied under positive pressure by a pump, dried with Drierite®, metered (±1%) through Sensirion or Tylan mass flow controllers (Staefa, Switzerland and Billerica, MA, USA, respectively) and routed through the animal chambers (see below). Flow controllers were calibrated at the test sites against a dry volume meter (Singer DTM-115; American Meter Company, Horsham, PA, USA). Excurrent air from the chambers was sub-sampled at 100–150 ml min^{-1}, dried and analyzed for oxygen content with a Sable Systems Oxzilla (Las Vegas, NV, USA). Instrument outputs were digitized by Sable Systems UI-2 or National Instruments PCI-MIO-16XE-50 (Austin, TX, USA) A-D converters and recorded by Macintosh computers running LabHelper software (www.warthog.ucr.edu).

Maximum oxygen consumption
Maximum oxygen consumption ($\dot{V}_{O_2,max}$) was measured during forced exercise in enclosed running wheel respirometry chambers. Air entered and exited the wheels though two airtight axial bearings. A manifold on the incurrent side dispersed flow and assisted gas mixing, which was also facilitated by animal motion and wheel rotation. We tested ground squirrels in a large wheel (32 cm diameter × 11 cm wide; internal volume about 9 liters) at a flow rate of 5.0 l min^{-1} (standard temperature and pressure, STP). For chipmunks, we used a smaller wheel (16.5 cm diameter × 7 cm wide, internal volume about 1.5 liters) at flows of 2.0 or 2.5 l min^{-1} STP. Subsampled excurrent air was dried (Drierite®), scrubbed of CO_2 (soda lime) and redried prior to O_2 content measurement. We sampled O_2 concentration and flow rate every 1.0 s.

To measure $\dot{V}_{O_2,max}$, we weighed animals (±0.1 g), sealed them into the wheel and took a reference reading of unbreathed air. With the wheel locked, we recorded $\dot{V}_{O_2}$ for several minutes while animals explored and acclimated to the chamber. Wheel rotation was initiated at low r.p.m. when animals were oriented in the appropriate direction. Most individuals immediately began walking or running to match wheel motion, and we increased rotation speed approximately every 30 s while monitoring behavior and $\dot{V}_{O_2}$. Rotation was stopped when animals were no longer able to maintain position or $\dot{V}_{O_2}$ did not increase with increasing speed. At this point, most exhibited obvious behavioral signs of exhaustion (panting, cessation of movement) but none showed indications of hyperthermia (salivation, licking the forelimbs, etc.). We recorded $\dot{V}_{O_2}$ for several minutes during the post-exercise recovery period and then took a second reference reading and removed the animal. All tests were performed at room temperature (22–25°C) during the normal diurnal activity period of the two species (there were no significant temperature differences among species or locations).

The Mode 1 equation in Warthog LabAnalyst (www.warthog.ucr.edu) was used to convert O_2 concentrations to $\dot{V}_{O_2}$ as:

$$\dot{V}_{O_2} = F \times (F_{I_{O_2}} - F_{E_{O_2}}) / (1 - F_{E_{O_2}}), \tag{1}$$

where F is flow rate (STP corrected), and $F_{I_{O_2}}$ and $F_{E_{O_2}}$ are incurrent (reference) and excurrent fractional O_2 concentrations, respectively ($F_{I_{O_2}}$ was assumed to be 0.2095). Since $\dot{V}_{O_2}$ usually did not attain steady state during forced-exercise tests, we used the 'instantaneous' correction to compensate for mixing and to resolve short-term metabolic changes (Bartholomew et al., 1981). Effective volumes, determined from washout kinetics, were 900 ml for the small wheel and 8300 ml for the large wheel (they were similar at both altitudes). We calculated $\dot{V}_{O2,max}$ as the highest 1 min running average of $\dot{V}_{O_2}$ during exercise.

Voluntary wheel-running

To determine behavior and energy costs during voluntary running, we used enclosed running wheel respirometers that permitted simultaneous measurement of speed and $\dot{V}_{O_2}$. In brief, a 1.12 m circumference rodent wheel constructed of stainless steel and acrylic plastic (Lafayette Instruments, Lafayette, IN, USA) was enclosed in a Plexiglas housing [fig. 1 in Chappell et al. (Chappell et al., 2004)]. The enclosure also contained a polycarbonate mouse cage (27.5 cm×17 cm×12 cm) with bedding, a drinking tube and a food hopper containing rodent chow. Dry food was supplemented with grapes or apple chunks. A 7.7 cm-diameter port in the cage wall let animals move freely between cage and wheel. The speed and direction of wheel rotation were transduced by a tachometer, and an internal fan rapidly circulated and mixed air. Air temperature in the wheel enclosures was measured with a thermocouple thermometer and ranged between 18 and 29°C (cooler at night and warmer during the day). Measurements were performed at the prevailing ambient photoperiod (approximately 13 h:11 h L:D).

Enclosures were supplied with dry air at flow rates of 2500 ml min^{-1} STP by a pump and mass flow controller as described above for $\dot{V}_{O2,max}$ measurements. Wheel speed and direction, O_2 concentration, chamber temperature, and flow rate were recorded every 1.5 s, and a computer-driven solenoid system (Sable Systems multiplexer) obtained 2-min reference readings every 45 min. Voluntary activity tests lasted 23–24 h, so we did not remove CO_2 prior to O_2 analysis to avoid either frequent scrubber changes or large volumes of scrubber chemicals that would impede response time. Sub-sampled air was dried with magnesium perchlorate, and we used the Mode 2 equation in LabAnalyst to calculate $\dot{V}_{O_2}$:

$$\dot{V}_{O_2} = F \times (F_{I_{O_2}} - F_{E_{O_2}}) / [1 - F_{E_{O_2}} \times (1 - RQ)], \qquad (2)$$

where RQ is the respiratory quotient. We assumed a constant RQ of 0.85, which is appropriate for a mixed diet of carbohydrate, lipid and protein and is similar to measured RQ for rodents eating standard laboratory diets (Chappell et al., 2004). Use of constant RQ introduces errors if real RQ differs from the assumed value. However, these errors are quite small: at typical $F_{E_{O_2}}$ (0.204–0.2085 in these measurements), the maximum error in $\dot{V}_{O_2}$ estimates from Eqn 2 is about 3% for real RQ between 0.7 and 1.0. As for $\dot{V}_{O2,max}$, we applied the 'instantaneous' transformation to $\dot{V}_{O_2}$ during voluntary running (Bartholomew et al., 1981). Effective volume of the wheel enclosure was 17 liters at SNARL and 14 liters at Barcroft.

To determine the relationship between running speed and $\dot{V}_{O_2}$, we lag-corrected $\dot{V}_{O_2}$ by 40 s to synchronize the two parameters; this was necessary because the system instantly detected wheel speed but detection of changes in $\dot{V}_{O_2}$ was delayed due to the flux of air through the respirometry plumbing and the relatively slow response of the Oxzilla analyzer. Because successive 1.5 s readings of wheel speed and $\dot{V}_{O_2}$ are not independent (due to rotational momentum and system lag times as well as behavior and physiology), we used the LabAnalyst stepped sampling procedure to avoid autocorrelation

problems. This algorithm computed 1 min averages separated by 3 min; with this protocol there is no statistically significant correlation between sequential 1 min averages (Chappell et al., 2004; Rezende et al., 2005; Rezende et al., 2006) (and as tested for the species in the present study). All regressions were linear by visual inspection (e.g. Fig. 1). In addition to the speed *versus* metabolic rate relationship, we calculated several other behavioral and metabolic variables, including mean and maximal speeds, mean, minimal and maximal $\dot{V}_{O_2}$, and characteristics of running bouts (Table 1). We defined bouts as episodes of running where speed remained above 0.5 m min^{-1}.

The time in wheel respirometers varied somewhat among individuals (22–24.5 h), so we proportionally adjusted distance run per day (d_{run}), time spent running per day (t_{run}) and number of running bouts per day (N_{bout}) to a constant period of 24 h.

Statistics

Our main focus was the effects of altitude, rather than comparisons between the two species (Garland and Adolph, 1994), so most tests were within-species analysis of variance (ANOVA) or analysis of covariance (ANCOVA) with location (SNARL or Barcroft) as a fixed effect. For variables affected by body mass (e.g. $\dot{V}_{O_2}$), we included mass as a covariate. In some cases, we checked for species
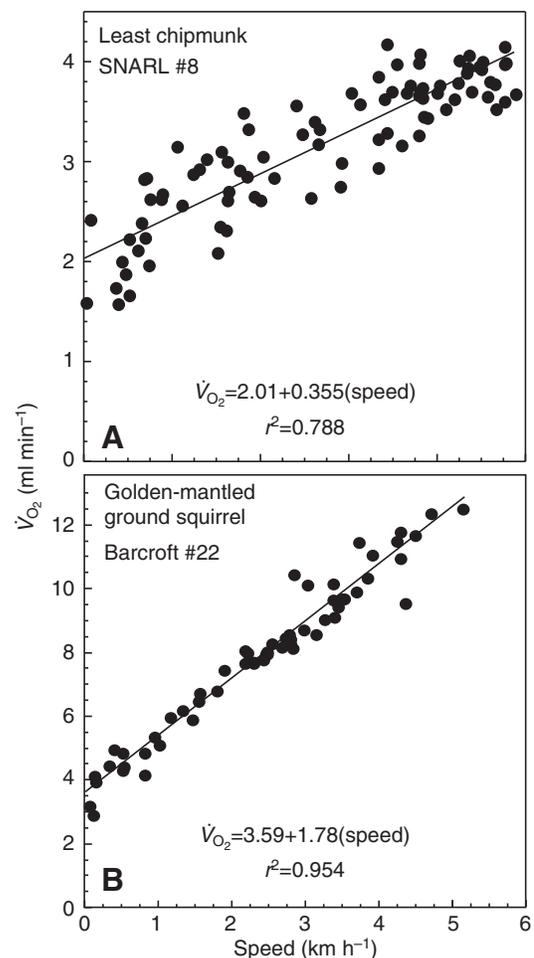

Fig. 1. Representative examples of relationships between wheel-running speed and metabolic rate in (A) a least chipmunk (30.9 g male measured at SNARL) and (B) a golden-mantled ground squirrel (144.3 g female measured at Barcroft).

Table 1. Metabolic and behavioral variables

Symbol	Description	Units
$\dot{V}_{O_2,max}$	Maximum $\dot{V}_{O_2}$ in forced exercise (1 min)	$ml\ O_2\ min^{-1}$
ADMR	Average daily metabolic rate	$kJ\ day^{-1}$
RMR	Minimal resting metabolic rate (10 min)	$ml\ O_2\ min^{-1}$
$\dot{V}_{O_2}1$	Maximum voluntary $\dot{V}_{O_2}$ averaged over 1 min	$ml\ O_2\ min^{-1}$
$\dot{V}_{O_2}2$	Maximum voluntary $\dot{V}_{O_2}$ averaged over 2 min	$ml\ O_2\ min^{-1}$
$\dot{V}_{O_2}5$	Maximum voluntary $\dot{V}_{O_2}$ averaged over 5 min	$ml\ O_2\ min^{-1}$
Slope	Slope of speed *versus* $\dot{V}_{O_2}$ regression	$ml\ O_2\ kg^{-1}\ km^{-1}$
Intercept	Intercept of speed *versus* $\dot{V}_{O_2}$ regression	$ml\ O_2\ kg^{-1}\ min^{-1}$
t_{run}	Time spent running per day	$min\ day^{-1}$
d_{run}	Distance run per day	$km\ day^{-1}$
V_{mean}	Mean voluntary running speed	$km\ h^{-1}$
V_{max}	Maximum voluntary running speed (1.5 s)	$km\ h^{-1}$
$V_{max}1$	Maximum voluntary running speed (1 min)	$km\ h^{-1}$
$V_{max}2$	Maximum voluntary running speed (2 min)	$km\ h^{-1}$
$V_{max}5$	Maximum voluntary running speed (5 min)	$km\ h^{-1}$
N_{bout}	Number of running bouts per day	
$Bout_{mean}$	Mean running bout duration	s
$Bout_{max}$	Maximum running bout duration	s

ADMR was computed from mean daily $\dot{V}_{O_2}$ assuming $20.1\ J\ ml^{-1}\ O_2$.

differences using ANOVA or ANCOVA with both location and species as fixed effects (interspecific differences in responses to altitude are indicated by significant species $\times$ location interaction terms). Because metabolic rate is a power function of mass, we used log_{10} values of mass and $\dot{V}_{O_2}$ in analyses (however, results are shown untransformed). In preliminary analyses, we tested for effects of age (juvenile or adult) and sex, but these were not significant after accounting for mass and were removed from the final models. To check for Type 1 errors in multiple tests, we computed false discovery rates (FDR) (Storey and Tibshirani, 2003; Storey, 2003). These tests were performed with the Qvalue library in the R statistical package (The R Foundation for Statistical Computing, Vienna, Austria) using the 'Bootstrap' option. Other analyses were performed with SPSS v.16 for the Macintosh (SPSS, Inc., Chicago, IL, USA).

RESULTS

We tested $\dot{V}_{O_2,max}$ on 22 golden-mantled ground squirrels at SNARL (111–160 g, seven males, 15 females) and 29 at Barcroft (119–293 g, 15 males, 14 females). Fourteen of the SNARL animals (six males, eight females) and 15 of the Barcroft animals (five males, 10 females) were also measured during voluntary activity. Thirty-two least chipmunks were tested for $\dot{V}_{O_2,max}$ at SNARL (28.2–39.5 g; 14 female, 18 male) and 15 were measured during voluntary activity (eight males, seven females). We tested $\dot{V}_{O_2,max}$ in 19 least chipmunks at Barcroft (26.0–38.2 g; 15 males, four females) and obtained voluntary activity data from seven of them (five males, two females).

In least chipmunks, there was no mass difference between the two sites (Table 2), but golden-mantled ground squirrels at SNARL averaged about 20% lighter than those at Barcroft (species $\times$ altitude interaction: $F=18.7$, $P<0.0001$). Nevertheless, even among ground squirrels there was considerable overlap in body mass at the two sites (Fig. 2).

Maximum oxygen consumption in forced exercise

Least chipmunks differed in $\dot{V}_{O_2,max}$ between SNARL and Barcroft ($P<0.0001$) (Table 2; Fig. 2). After correcting for body mass (ANCOVA with mass as covariate), $\dot{V}_{O_2,max}$ was about 36% higher at SNARL. By contrast, there was no significant difference in the mass-adjusted $\dot{V}_{O_2,max}$ of golden-mantled ground squirrels between the two sites ($P=0.96$) (Fig. 2; Table 2). The response to altitude differed significantly (species $\times$ altitude interaction: $F=13.3$, $P<0.0001$).

Metabolism during voluntary behavior

Ambient temperatures in the running wheel respirometers during the day (when all running behavior occurred) varied to some extent at both sites (20.5–29.2°C at SNARL; 20.5–27.2°C at Barcroft) but were usually within or close to the thermal neutral zone (Willems and Armitage, 1975; Heller and Gates, 1971; Heller and Poulson, 1972; Kenagy et al., 1989). There was no

Table 2. Metabolism (oxygen consumption, $\dot{V}_{O_2}$) during forced and voluntary exercise at the two test sites

Trait	Mean ± s.e.m. SNARL	Mean ± s.e.m. Barcroft	F mass	P mass	F altitude	P altitude
Least chipmunk						
Mass	32.0±0.47	31.3±0.71			0.52	0.48
$\dot{V}_{O_2,max}$	7.08±0.18	5.36±0.22	18.9	**<0.0001***	46.4	**<0.0001***
ADMR	49.5±1.7	46.5±2.9	0.60	0.45	0.63	0.44
RMR	0.742±0.045	0.791±0.069	0.88	0.36	0.33	0.57
$\dot{V}_{O_2}1$	4.88±0.30	5.38±0.45	10.9	**0.00376***	0.79	0.39
$\dot{V}_{O_2}2$	4.64±0.23	4.76±0.35	10.6	**0.00410***	0.08	0.79
$\dot{V}_{O_2}5$	4.20±0.15	3.83±0.23	4.67	**0.0437**	1.73	0.20
Golden-mantled ground squirrel						
Mass	131.7±5.9	163.3±5.1		16.3	**0.00018***	
$\dot{V}_{O_2,max}$	19.3±0.82	19.3±0.70	18.1	**<0.0001***	0.003	0.96
ADMR	165±9.0	131±8.7	12.4	**0.0016***	6.70	**0.016***
RMR	2.51±0.10	2.28±0.09	3.42	0.076	2.74	0.110
$\dot{V}_{O_2}1$	18.0±0.81	15.0±0.78	7.08	**0.0132***	6.45	**0.0174***
$\dot{V}_{O_2}2$	17.2±0.81	14.3±0.78	6.56	**0.0166***	5.93	**0.0221***
$\dot{V}_{O_2}5$	16.4±0.68	13.0±0.65	13.4	**0.00114***	12.2	**0.00173***

ANCOVA with altitude as a fixed effect and body mass as covariate.

For least chipmunks, sample sizes were 32 at SNARL (2165 m elevation) and 19 at Barcroft (3800 m) for $\dot{V}_{O_2,max}$, and 15 at SNARL and 7 at Barcroft for other traits. Sample sizes for golden-mantled ground squirrels were 22 at SNARL and 29 at Barcroft for $\dot{V}_{O_2,max}$, and 14 at SNARL and 15 at Barcroft for other traits.

All metabolic variables are shown after adjusted to common masses (for least chipmunks, 31.0 g for $\dot{V}_{O_2,max}$ and 33.6 g for other traits; for golden-mantled ground squirrels, 150.5 g for $\dot{V}_{O_2,max}$ and 145.5 g for other traits). Symbols and units as in Table 1. $P<0.05$ are in bold; P values that remained significant after an FDR test are indicated with asterisks (*).
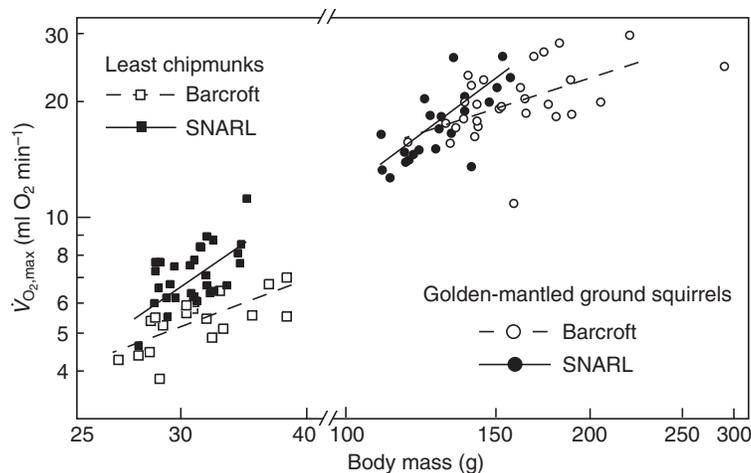
Fig. 2. Maximum metabolic rates ($\dot{V}_{O_2,max}$) during forced exercise in least chipmunks (left, squares) and golden-mantled ground squirrels (right, circles). Solid symbols and lines indicate measurements at SNARL (2165 m elevation), and open symbols and broken lines show measurements made at Barcroft (3800 m).

significant effect of temperature on any metabolic or behavioral variable.

Minimal resting metabolic rates (RMR) did not differ across altitude in either species (Table 2). In least chipmunks, there were no differences in any other metabolic index during voluntary behavior (ADMR and maximal $\dot{V}_{O_2}$ averaged over 1, 2 or 5 min) (Table 2). However, all of these indices were lower at Barcroft than at SNARL for golden-mantled ground squirrels (Table 2). For all these variables except RMR, the response to altitude was significantly different in the two species (species × altitude interaction: $F>4.6$, $P<0.037$).

Running behavior

There was no significant effect of altitude on either the time spent running or the total distance run in either species (Table 3). Individual maxima of d_{run} for least chipmunks were 21.7 km at Barcroft and 24.4 km at SNARL. The d_{run} individual maxima were somewhat higher for golden-mantled ground squirrels (34.2 km at Barcroft and 25.5 km at SNARL). Despite the statistical similarity of mean t_{run} and d_{run} across altitudes, in least chipmunks the average running speed (V_{mean}) was 38% faster at high altitude (Table 3) due to a shift in the frequency distribution of speeds (Fig. 3).

Table 3. Behavior and cost of transport during voluntary exercise over ~24 h at the two test sites, SNARL (2165 m elevation) and Barcroft (3800 m elevation)

Trait	Mean ± s.e.m. SNARL	Mean ± s.e.m. Barcroft	F mass	P mass	F altitude	P altitude
Least chipmunk						
Slope	1091±104	664±159	0.31	0.59	4.62	**0.0447**
Intercept	68.7±4.2	57.2±6.3	0.31	0.59	2.1	0.16
t_{run}	197±30.3	185±46.1	3.52	0.076	0.04	0.84
d_{run}	7.46±1.68	10.1±2.56	1.67	0.21	0.68	0.42
V_{mean}	2.12±0.14	2.93±0.22	0.37	0.55	8.81	**0.00790***
V_{max}	5.54±0.26	5.45±0.40	1.3	0.27	0.03	0.86
$V_{max}1$	4.12±0.23	4.76±0.35	0.07	0.80	2.1	0.16
$V_{max}2$	3.79±0.23	4.62±0.36	0.30	0.59	3.4	0.080
$V_{max}5$	3.28±0.27	4.43±0.41	0.23	0.64	5.04	**0.0371**
N_{bout}	281±38	220±58	0.46	0.51	0.74	0.40
$Bout_{mean}$	42.7±5.4	47.7±8.2	5.32	**0.0326**	0.24	0.63
$Bout_{max}$	431±108	771±164	0.03	0.88	2.7	0.11
Golden-mantled ground squirrel						
Slope	592±37	667±35	2.1	0.16	2.0	0.17
Intercept	52.6±2.3	37.6±2.2	0.63	0.44	19.7	**0.000149***
t_{run}	199±30.3	203±29.1	2.8	0.11	0.009	0.93
d_{run}	12.7±2.25	13.1±2.17	4.0	0.055	0.009	0.93
V_{mean}	3.92±0.22	3.36±0.22	6.23	**0.0193**	2.9	0.098
V_{max}	8.28±0.38	8.52±0.36	0.07	0.79	0.19	0.67
$V_{max}1$	6.57±0.36	6.18±0.35	0.12	0.73	0.57	0.46
$V_{max}2$	6.12±0.33	5.69±0.32	0.14	0.71	0.82	0.37
$V_{max}5$	5.68±0.35	4.85±0.34	1.1	0.30	2.6	0.12
N_{bout}	349±49	270±47	2.7	0.11	1.2	0.28
$Bout_{mean}$	42.7±4.9	42.8±4.7	0.01	0.91	0	0.99
$Bout_{max}$	444±80	347±74	0.38	0.54	0.70	0.41

ANCOVA with altitude as a fixed effect and body mass as covariate.
Symbols and units as in Table 1; sample sizes as in Table 2. $P<0.05$ are in bold; P values that remained significant after an FDR test are indicated with asterisks (*).
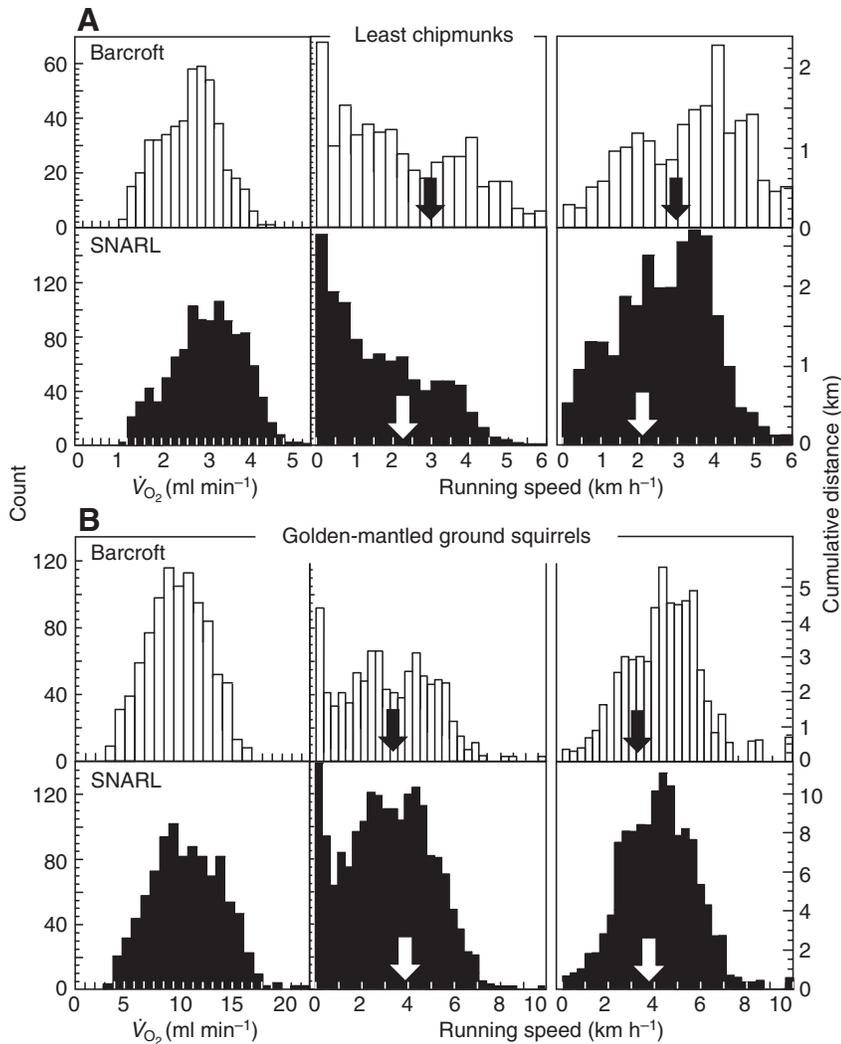
Fig. 3. Frequency distributions of $\dot{V}_{O_2}$ during running (left) and running speed (center), and the distance traveled at different speeds (right) for (A) least chipmunks and (B) golden-mantled ground squirrels during voluntary locomotion in running wheels. Filled histograms show data obtained at SNARL (2165 m elevation), and open histograms show data obtained at Barcroft (3800 m). Downward-pointing arrows show mean speeds.

Maximum instantaneous speed (V_{max}) was not affected by altitude in either species, and the maximum speed averaged over 1, 2 and 5 min was unaffected by altitude in golden-mantled ground squirrels. In least chipmunks, $V_{max}5$ was significantly faster (by 35%) at Barcroft than at SNARL, but none of the other maximal speed measures differed across altitudes.

The number, mean duration and maximum duration of running bouts was not affected by altitude in either species. Frequency distributions of speed and $\dot{V}_{O_2}$ during running revealed generally similar overall patterns at SNARL and Barcroft for both species (Fig. 3). However, there was a substantial upward shift in running speeds of least chipmunks at Barcroft, with a greater fraction of total distance run at speeds higher than about 3 km h^{-1}.

Energy costs of running

The relationship between speed and metabolic rate was always significantly positive in both species, but r^2 values tended to be higher for golden-mantled ground squirrels than for least chipmunks (e.g. Fig. 1). Mass-specific energy costs of running of both species, estimated from regression slopes and intercepts, tended to be lower at high altitude (Fig. 4). However, the reduction in running costs occurred in different ways. In least chipmunks, the regression slope (the 'instantaneous cost of transport', iCOT) was lower at Barcroft than at SNARL (although significance was marginal) but there was no difference in intercept (Table 3; Fig. 4). Thus, based on mean

iCOT and intercept, running costs were similar at low speeds, but running became less costly at high altitude than at low altitude as speed increased. By contrast, golden-mantled ground squirrels had similar iCOT but different intercepts at the two altitudes (Table 3; Fig. 4); again, the total cost of running estimated from mean iCOT and intercept values was lower at high altitude, but in this species the relative cost difference declined as speed increased. As expected, intercepts were significantly higher than RMR ($P<0.0001$ in all combinations of species and altitude, paired t-tests).

To determine if voluntary running was within aerobic limits, we first compared forced-exercise $\dot{V}_{O_2,max}$ with the highest mean $\dot{V}_{O_2}$ attained during voluntary exercise. Except for SNARL golden-mantled ground squirrels, $\dot{V}_{O_2,max}$ was always significantly higher than $\dot{V}_{O_2}1$, and without exception $\dot{V}_{O_2,max}$ was significantly higher than $\dot{V}_{O_2}2$ and $\dot{V}_{O_2}5$ (Table 4). Thus, the two species usually stayed within aerobic limits even during the highest voluntary speeds. Second, we used pooled $\dot{V}_{O_2,max}$ and cost of transport data to estimate the maximum aerobic speed (MAS) for each species and altitude as MAS=($\dot{V}_{O_2,max}$–intercept)/iCOT. In least chipmunks, MAS was 8.73 km h^{-1} at SNARL and 10.3 km h^{-1} at Barcroft; all voluntarily attained speeds were well below these values (Table 3; Fig. 3). For golden-mantled ground squirrels, MAS was 9.5 km h^{-1} at SNARL and 7.3 km h^{-1} at Barcroft. In this species, as for least chipmunks, all voluntary maximal speeds averaged over 1, 2 or 5 min were substantially below MAS. However, at Barcroft, the maximum
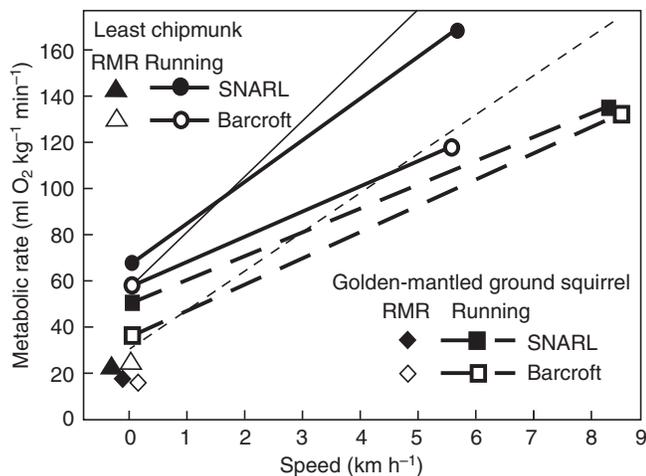
Fig. 4. The energy cost of transport in least chipmunks (circles and solid lines) and golden-mantled ground squirrels (squares and broken lines). The lines were generated from mean values of the slope and intercept of the speed *versus* $\dot{V}_{O_2}$ relationships. Filled symbols indicate values measured at SNARL (2165 m elevation), and open symbols indicate values measured at Barcroft (3800 m). Lines terminate at the highest observed running speed (1-min average) at each site. Triangles and diamonds are means of minimal resting metabolic rates (RMR) for least chipmunks and golden-mantled ground squirrels, respectively. Thin lines are predictions from the Taylor et al. (Taylor et al., 1982) allometric equations for metabolic rate during running (solid line=33.6 g least chipmunk, and broken line=144.5 g golden-mantled ground squirrel).

instantaneous speed (V_{max}, the fastest speed in a 1.5 s measurement interval) averaged 1.2 km h^{-1} faster than MAS. Thus, these squirrels occasionally sprinted anaerobically for brief periods.

DISCUSSION

Our main goal was to determine if a substantial difference in altitude affected aerobic capacity and running performance in wild populations. Any study of this type should consider the natural altitude distribution of the tested species. In central California, golden-mantled ground squirrels and least chipmunks both occur at somewhat lower altitudes than SNARL [the lower limit in this region over the past century was about 1500 m (Hall, 1981); current lower limits for *S. lateralis* are closer to 2000 m (Moritz et al., 2008)]. The Barcroft Laboratory is approximately at the upper altitude limit for least chipmunks (Hall, 1981); in the White Mountains they were historically found no higher than about 3600 m and were first noticed regularly by researchers and station staff at Barcroft (3800 m) within the last several years (M.A.C. and D. Cann, personal observations).

We have not seen them at higher altitudes in the White Mountains. However, golden-mantled ground squirrels occasionally occur up to the highest point in this range (White Mountain Peak, 4342 m; M.A.C., personal observations). During the study, both species were common at SNARL and Barcroft.

We assumed that any effects of altitude on performance would result from differences in oxygen availability. The effects of declining pressure with increased elevation on P_{O_2} are enhanced by the dilution of inspired air by water evaporated from the respiratory tract. Barometric pressure during our field work averaged 585 torr at SNARL and 474 torr at Barcroft; thus, ambient P_{O_2} in dry air was 122.6 and 99.3 torr, respectively. Assuming a body (and alveolar) temperature of 37.5°C and 100% saturation of respiratory gas spaces with water vapor, the maximum P_{O_2} in inspired air was 112.3 torr at SNARL and 89.0 torr at Barcroft (for comparison, inspired P_{O_2} at sea level at the same body temperature is about 149 torr).

Aerobic capacity and altitude

Did the 26% lower inspired P_{O_2} at Barcroft suppress aerobic capacity or voluntary running? The answer for aerobic capacity ($\dot{V}_{O_2,max}$) is mixed. In least chipmunks, $\dot{V}_{O_2,max}$ at Barcroft was lower than at SNARL by about 36% (Table 2), considerably more than the difference in inspired P_{O_2}. By contrast, the $\dot{V}_{O_2,max}$ of golden-mantled ground squirrels was unaffected by the altitude difference. We are not aware of any published values for exercise-induced $\dot{V}_{O_2,max}$ at low altitudes in these two species, but there are some data for congeners. Hoyt and Kenagy (Hoyt and Kenagy, 1988) estimated the $\dot{V}_{O_2,max}$ of cascade golden-mantled ground squirrels (*S. saturatus*, mean body mass about 230 g), which are very closely related to *S. lateralis*. Their reported $\dot{V}_{O_2,max}$ for *S. saturatus* was 10 ml O$_2$ g^{-1} h^{-1}, tested approximately at sea level (D. Hoyt, personal communication). Our results for *S. lateralis* are about 21% lower. Assuming that the aerobic physiologies of *S. saturatus* and *S. lateralis* are similar and the effects of the body mass difference are minor, these data suggest $\dot{V}_{O_2,max}$ at our sites was reduced compared to that at sea level but – at least at Barcroft – there was considerable compensation for the 41% difference in inspired P_{O_2}. Wunder ran Merriam's chipmunks (*T. merriami*, body mass 75 g) on a treadmill at low altitude (Los Angeles, CA, USA) and attained a maximum $\dot{V}_{O_2}$ of 7.07 ml O$_2$ g^{-1} h^{-1}, or ~8.8 ml min^{-1} (Wunder, 1970). That is 20–39% higher than what we observed for *T. minimus* at SNARL and Barcroft, respectively, and suggests little compensation for hypoxia in *Tamias*.

Voluntary running and altitude

The ability to move around the habitat in 'routine' activities such as finding food and mates or patrolling territories is probably at

Table 4. Comparison of forced-exercise $\dot{V}_{O_2,max}$ with maximal $\dot{V}_{O_2}$ during voluntary running at the two test sites, SNARL (2165 m elevation) and Barcroft (3800 m elevation)

Altitude	$\dot{V}_{O_2}1$		$\dot{V}_{O_2}2$		$\dot{V}_{O_2}5$	
	F	P	F	P	F	P
Golden-mantled ground squirrel						
2165	2.76	0.106	5.66	**0.023**	23.9	**<0.0001**
3800	18.0	**0.000124**	26.0	**<0.0001**	8.96	**0.00584**
Least chipmunk						
2165	91.4	**<0.0001**	113	**<0.0001**	151	**<0.0001**
3800	4.65	**0.0417**	13.3	**0.00131**	53.0	**<0.0001**

Symbols and units as in Table 1; sample sizes as in Table 2. $P<0.05$ in bold, and all remained significant after an FDR test.

Table 5. Energy expended in wheel-running on a daily basis, computed from mean values in Tables 2 and 3

Altitude	Minimal COT	Postural costs	Total COT
Golden-mantled ground squirrel			
2165	1006 (12.3%)	880 (10.7%)	1886 (23.0%)
3800	1427 (21.9%)	784 (12.0%)	2211 (33.9%)
Least chipmunk			
2165	260.4 (10.6%)	286.8 (11.5%)	547.2 (22.2%)
3800	209.9 (9.1%)	184.9 (8.0%)	394.8 (17.1%)

Minimal COT=iCOT×d_{run}; postural costs=intercept–RMR; total COT=minimal COT+postural costs. Units are ml O_2 day^{-1}; values in parentheses are percentages of daily energy expenditures.

least as important to fitness as high $\dot{V}_{O2,max}$. It is difficult to measure the distance, duration and, particularly, the energy cost of natural locomotion in free-living animals, but our voluntary wheel-running results offer several useful insights. Wheel-running was extensive at SNARL and Barcroft (averaging 7.5–13 km day^{-1}), and we found little evidence of a detrimental or inhibitory effect of high altitude. In both golden-mantled ground squirrels and least chipmunks, the amount of time spent running per day and the total distance traveled did not differ significantly between the two sites. Surprisingly, least chipmunks – which had substantially reduced $\dot{V}_{O2,max}$ at Barcroft – ran at a higher mean speed there than at SNARL (Table 3; Fig. 3). Other indices of running endurance, such as the mean maximal speeds averaged over 1, 2 and 5 min, and the mean and maximal duration of running bouts, either were not significantly affected by altitude or, in some cases, were higher in the lower P_{O2} at Barcroft ($V_{max}5$ for least chipmunks) (Table 3).

Running in wheels probably involves different motivational factors and possibly has different energy costs from those of free-living animals traveling on natural terrain (see below). Nevertheless, our findings suggest that normal locomotor behavior of both species is unlikely to be inhibited by the reduced oxygen availability at Barcroft. We do not know the magnitude or speed of daily movements in wild ground squirrels or chipmunks at our study sites, but free-living cascade golden-mantled ground squirrels in a Washington State conifer forest (altitude 600–675 m) (Kenagy and Hoyt, 1989; Kenagy et al., 1989) traveled an average of 5.0 km day^{-1}. That is less than half of the mean distance run in wheels by our squirrels. However, the *S. saturatus* in Kenagy and Hoyt's study often ran faster than our animals, typically traveling at about 13 km h^{-1}. Part of the difference may be due to size (the *S. saturatus* averaged 230 g compared with ~150 g for our *S. lateralis*), but other factors (possibly including wheel characteristics) are likely to be important as well. Subjectively, speeds used by undisturbed individuals of our two species in the wild appeared to be considerably less than 13 km h^{-1}, and both frequently walked at slow speeds.

If altitude hypoxia did not constrain voluntary running, what was the limiting factor? Several possible causes merit consideration, including limits to the time available for running, hyperthermia due to exercise, and muscle or organ hypoxia. Time limitations were probably not a factor. Both species ran exclusively between sunrise and sunset, but for all individuals the time spent running was much less than the duration of daylight. Exercise-induced hyperthermia can limit performance in warm conditions in large animals such as humans, and artificially augmented heat loss can enhance performance (Grahn et al., 2005). Could the running behavior of our animals have been limited by heat build-up? That explanation is attractive because exercise hyperthermia – like voluntary running – should be largely independent of altitude, but we think it unlikely

for several reasons. First, $\dot{V}_{O2,max}$ tests elicited higher metabolic rates (and hence heat production) than voluntary running but animals maintained very high power output for 4–10 min, much longer than the duration of most voluntary running bouts (Table 3). Despite their high power output in $\dot{V}_{O2,max}$ tests, our animals showed no signs of heat stress; substantial hyperthermia in sciurid rodents elicits extensive salivation over the chin, throat and forelimbs to enhance evaporative cooling (e.g. Wunder, 1970). Second, we found no relationship between bout duration (or any other behavioral variable) with ambient temperature. Third, maximum bout lengths were many times greater (by 8 to 16-fold) (Table 3) than mean bout duration despite being run at similar speeds; if hyperthermia were a major limiting factor for routine running we would expect few bouts to be substantially longer than mean bout duration.

Hypoxia within specific high-activity organs (skeletal muscles, heart, brain, etc.) has been proposed as a limiting factor for exercise, perhaps under control of a central 'governor' (e.g. Noakes et al., 2001). This also seems unlikely to explain limits to wheel-running in our animals for many of the reasons described above; oxygen use in $\dot{V}_{O2,max}$ tests was both more intense and considerably longer than during voluntary running bouts, and the occurrence of some bouts many-fold longer than mean bout duration suggests that the latter were not hypoxia-limited. Also, a hypoxic limitation to exercise is not consistent with lack of altitude effects on exercise. We speculate that motivational factors, rather than physiological constraints, controlled the intensity and duration of voluntary activity in our squirrels and chipmunks.

Energy cost of transport

One interesting finding was the apparent reduction in costs of transport at high altitude in both least chipmunks and golden-mantled ground squirrels (Table 3; Fig. 4). The somewhat lower intercept at Barcroft for golden-mantled ground squirrels likely had little impact on either energy costs or running ability, since predicted metabolic rates at high speeds were quite similar for the two study sites. However, in least chipmunks, the lower iCOT at Barcroft resulted in a substantially lower predicted energy cost of running at high speed compared with chipmunks running at SNARL (Fig. 4). Did this decrease in transport cost allow chipmunks at Barcroft to run voluntarily at higher speeds than would otherwise have been possible? Probably not: even if the Barcroft chipmunks had the same transport costs as those at SNARL, predicted $\dot{V}_{O2}$ during the highest 1-, 2- and 5-min voluntary running speeds (4.4–4.8 km h^{-1}) (Table 3; Fig. 4) were less than $\dot{V}_{O2,max}$. At both altitudes, the estimated maximal aerobic speed for least chipmunks was always greater than voluntarily achieved speeds; thus, there was little indication that aerobic physiology limited voluntary running performance.

Running energetics in mammals has been extensively studied for decades (e.g. Wunder 1970; Taylor et al., 1970; Taylor et al., 1982;

Tucker, 1975). How do our measured costs of transport compare with COT for other species? Such comparisons are both interesting and problematic because the data were obtained in quite different ways. Our measurements were of voluntary wheel-running by freshly captured wild individuals and were characterized by highly intermittent running bouts that included a range of speeds and typically short but highly variable duration (Table 3). Animals could run uphill, downhill or on the level in wheels and often changed position numerous times in a single bout of running. By contrast, almost all published data on COT – particularly for small mammals – were obtained from captive animals forced to run on level treadmills at constant speeds for relatively long periods (typically, many minutes), usually after extensive training.

Which of the two approaches is most realistic for estimating COT in free-living animals moving over complex terrain (as is typical of our study sites) remains an open question. However, in both golden-mantled ground squirrels and least chipmunks, the running-wheel data yielded lower iCOT than an allometric equation [eqn 9 in Taylor et al. (Taylor et al., 1982)] predicting $\dot{V}_{O_2}$ from speed and body mass (Fig. 4). Although intercepts derived from allometry tended to be less than what we observed, at all but the lowest speeds the total energy cost during running in our two species was less than predicted. In the only species for which treadmill and wheel-running COT were measured in the same individuals (Mongolian gerbils, *Meriones unguiculatus*) (Chappell et al., 2007b), voluntary wheel-running COT was also lower than treadmill COT. However, in gerbils the low COT in voluntary exercise was due to a substantially lower intercept compared with forced exercise values instead of a reduced iCOT.

Hoyt and Kenagy (Hoyt and Kenagy, 1989) measured COT in cascade golden-mantled ground squirrels, and Wunder (Wunder, 1970) measured COT in Merriam's chipmunks. Both used treadmill exercise and both of these species were larger than the congeners we studied. In *S. saturatus*, total COT was higher than predicted by the Taylor et al. (Taylor et al., 1982) equation, except at the highest speeds [fig. 2 in Hoyt and Kenagy (Hoyt and Kenagy, 1988)]. This contrasts with our finding of total COT lower than predicted at high speeds (Fig. 4), and the overall slope for *S. saturatus* (walking + running combined; ~697 ml O$_2$ kg^{-1} km^{-1}) was somewhat greater than iCOT in our *S. lateralis* (Table 3). The *S. saturatus* also showed a distinct effect of speed and gait on iCOT, with walking having a higher iCOT than running and a noticeable overall inflection in the overall speed *versus* $\dot{V}_{O_2}$ relationship at about 3 km h^{-1}. We did not notice inflections in our running wheel data (e.g. Fig. 1). It is not clear whether the contrast between the two studies is due to the biomechanical aspects of running on a treadmill *versus* running in a wheel, motivational differences between forced *versus* voluntary running, or other factors. In Merriam's chipmunks, intercepts were approximately as predicted by the Taylor et al. (Taylor et al., 1982) equation, but slopes [computed from the 30°C data in table 2 of Wunder (Wunder, 1970)] were somewhat lower. The *T. minimus* in our study had higher intercepts than *T. merriami* (unsurprising considering that the latter is twice as heavy as *T. minimus*), but slopes were similar in the two species (885 for *T. merriami versus* 664 and 1091 ml O$_2$ kg^{-1} km^{-1} for *T. minimus*). Thus, there is no consistent pattern in forced *versus* voluntary COT among these species.

The fraction of daily energy expenditure (DEE) used to power running, or the 'ecological cost of transport' (ECT), is of interest to behavioral ecologists as well as exercise physiologists (e.g. Garland, 1983). It can be computed as the minimal COT to move the mass of the animal over its daily movement distance as iCOT

$\times d_{run}$. An alternative measure of transport expenses is the total COT, equivalent to iCOT $\times d_{run}$ plus the 'postural cost', which is equal to the time spent moving multiplied by the difference between the speed *versus* $\dot{V}_{O_2}$ intercept and RMR: t_{run}(intercept–RMR). Allometric analyses (Garland, 1983) indicate that ECT is insignificant for small mammals, primarily because the estimated daily movement distance in the Garland (Garland, 1983) study was quite small. Our values for minimal COT (9–22% of DEE) and total COT (including postural costs; 17–34% of DEE) are much larger than predictions from allometry. They are also larger than those reported for free-living *S. saturatus* (total COT of 13% of DEE) (Kenagy and Hoyt, 1989) and for voluntary wheel-running by deer mice (minimal COT of ~6% of DEE) (Chappell et al., 2004) and laboratory mice (*Mus domesticus*; minimal COT of 4.4–7.5% of DEE) (Koteja et al., 1999). In large part, the higher COT in our two sciurids stems from their extensive running behavior; both least chipmunks and golden-mantled ground squirrels ran considerably further per day than did *S. saturatus* deer mice or laboratory mice.

In summary, we did not find consistent effects of a 1.6 km difference in altitude, and the corresponding 26% change in inspired P_{O_2}, on either aerobic capacity or voluntary exercise. The altitude gradient had a strong influence on aerobic capacity ($\dot{V}_{O_2,max}$) in least chipmunks but not in golden-mantled ground squirrels. We found no affect of altitude on the distance or duration of voluntary running in either species, despite the 36% reduction in the $\dot{V}_{O_2,max}$ of least chipmunks at the high-altitude site. Most voluntary running was well within aerobic limits, although golden-mantled ground squirrels at the high-altitude site occasionally performed brief anaerobic sprints. Our findings indicate that species or populations native to high altitudes do not necessarily suffer reduced aerobic capacity compared with lower-elevation conspecifics and, even if they do, the scope of voluntary locomotion may not be impacted. Thus, altitude hypoxia may have little direct impact on physiology, behavior or ecology in these two species, although other aspects of life at high altitudes – low temperatures, long winters, low productivity, etc. – may be of considerable physiological and ecological importance.

Finally, it is worth noting that our data may have some relevance for the potential of mammals in mountainous regions to withstand global climate change. A recent study of small mammal distributions in the central Sierra Nevada region (very close to SNARL) found that, over the past century, altitude limits have moved upwards by an average of 500 m and for some species by as much as 1 km (Moritz et al., 2008). This upward distributional shift has the potential to put some species – especially high-altitude forms – at risk because of range contraction, but our results suggest that it probably will not lead to hypoxic limitations to routine behavior.

REFERENCES

Bartholomew, G. A., Vleck, D. and Vleck, C. M. (1981). Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturnid moths. *J. Exp. Biol.* **90**, 17-32.
Chappell, M. A., Garland, T., Jr, Rezende, E. L. and Gomes, F. R. (2004). Voluntary running in deer mice: speed, distance, energy costs, and temperature effects. *J. Exp. Biol.* **207**, 3839-3854.

Chappell, M. A., Hammond, K. A., Cardullo, R. A., Russell, G. A., Rezende, E. L. and Miller, C. (2007a). Deer mouse aerobic performance across altitudes: effects of developmental history and temperature acclimation. *Physiol. Biochem. Zool.* **80**, 652-662.

Chappell, M. A., Garland, T., Robertson, G. F. and Saltzman, W. (2007b). Relationships among running performance, aerobic physiology and organ mass in male Mongolian gerbils. *J. Exp. Biol.* **210**, 4179-4197.

Cymerman, A., Reeves, J. T., Sutton, J. R., Rock, P. B., Groves, B. M., Malconian, M. K., Young, P. M., Wagner, P. D. and Houston, C. S. (1989). Operation Everest II: maximal oxygen uptake at extreme altitude. *J. Appl. Physiol.* **66**, 2446-2453.

Garland, T., Jr (1983). Scaling the ecological cost of transport to body mass in terrestrial mammals. *Am. Nat.* **121**, 571-587.

Garland, T., Jr and Adolph, S. C. (1994). Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiol. Zool.* **67**, 797-828.

Gonzales, N. C., Clancy, R. L. and Wagner, P. D. (1993). Determinants of maximal oxygen uptake in rats acclimated to simulated altitude. *J. Appl. Physiol.* **75**, 1608-1614.

Grahn, D. A., Cao, V. H. and Heller, H. C. (2005). Heat extraction through the palm of one hand improves aerobic exercise endurance in a hot environment. *J. Appl. Physiol.* **99**, 972-978.

Hall, E. R. (1981). *The Mammals of North America*, 2nd edn. New York: John Wiley.

Hayes, J. P. (1989a). Field and maximal metabolic rates of deer mice (*Peromyscus maniculatus*) at low and high altitudes. *Physiol. Zool.* **62**, 732-744.

Hayes, J. P. (1989b). Altitudinal and seasonal effects on aerobic metabolism of deer mice. *J. Comp. Physiol.* **159B**, 453-459.

Heller, H. C. and Gates, D. M. (1971). Altitudinal zonation of chipmunks (*Eutamias*) energy budgets. *Ecology* **52**, 424-433.

Heller, H. C. and Poulson, T. (1972). Altitudinal zonation of chipmunks (*Eutamias*) adaptations to aridity and high temperature. *Am. Midl. Nat.* **87**, 296-313.

Hoyt, D. F. and Kenagy, G. J. (1988). Energy costs of walking and running gaits and their aerobic limits in golden-mantled ground squirrels. *Physiol. Zool.* **61**, 34-40.

Huey, R. B., Salisbury, R., Wang, J. L. and Mao, M. (2007). Effects of age and gender on success and death of mountaineers on Mount Everest. *Biol. Lett.* **3**, 498-500.

Kenagy, G. J. and Hoyt, D. F. (1988). Energy cost of walking and running gaits and their aerobic limits in golden-mantled ground squirrels. *Physiol. Zool.* **61**, 34-40.

Kenagy, G. J. and Hoyt, D. F. (1989). Speed and time-energy budget for locomotion in golden-mantled ground squirrels. *Ecology* **70**, 1834-1839.

Kenagy, G. J., Sharbaugh, S. M. and Nagy, K. A. (1989). Annual cycle of energy and time expenditure in the golden-mantled ground squirrel. *Oecologia* **78**, 269-282.

Koteja, P., Swallow, J. G., Carter, P. A. and Garland, T., Jr (1999). Energy cost of wheel-running in house mice: implications for coadaptation of locomotion and energy budgets. *Physiol. Biochem. Zool.* **72**, 238-249.

Lindstedt, S. L. and Conley, K. E. (2001). Human aerobic performance: too much ado about limits to $\dot{V}_{O_2}$. *J. Exp. Biol.* **204**, 3195-3199.

Moritz, C., Patton, J. L., Conroy, C. J., Parra, J. L., White, G. C. and Beissinger, S. R. (2008). Impact of a century of climate change on small-mammal communities in Yosemite National Park, USA. *Science* **322**, 261-264.

Noakes, T. D., Peltonen, J. E. and Rusko, H. K. (2001). Evidence that a central governor regulates exercise performance during acute hypoxia and hyperoxia. *J. Exp. Biol.* **204**, 3225-3234.

Rezende, E. L., Chappell, M. A., Gomes, F. R., Malisch, J. L. and Garland, T., Jr (2005). Maximal metabolic rates during voluntary exercise, forced exercise, and cold exposure in house mice selectively bred for high wheel-running. *J. Exp. Biol.* **208**, 2447-2458.

Rezende, E. L., Kelly, S. A., Gomes, F. R., Chappell, M. A. and Garland, T., Jr (2006). Effects of size, sex, and voluntary running speeds on costs of locomotion in lines of laboratory mice selectively bred for high wheel-running activity. *Physiol. Biochem. Zool.* **79**, 83-99.

Storey, J. D. (2003). The positive false discovery rate: a Bayesian interpretation and the q-value. *Ann. Stat.* **31**, 2013-2035.

Storey, J. D. and Tibshirani, R. (2003). Statistical significance for genome-wide experiments. *Proc. Nat. Acad. Sci. USA* **100**, 9940-9445.

Taylor, C. R., Schmidt-Nielsen, K. and Raab, J. L. (1970). Scaling of the energetic cost of running to body size in mammals. *Am. J. Physiol.* **219**, 1104-1107.

Taylor, C. R., Heglund, N. and Maloiy, G. M. O. (1982). Energetics and mechanics of terrestrial locomotion. I. Metabolic energy consumption as a function of speed and body size in birds and mammals. *J. Exp. Biol.* **97**, 1-21.

Tucker, V. A. (1975). The energetic cost of moving about. *Am. Sci.* **63**, 413-419.

Weibel, E. R. (1984). *The Pathway for Oxygen*. Cambridge, MA: Harvard University Press.

West, J. B. (2006). Human responses to extreme altitudes. *Integr. Comp. Biol.* **46**, 25-34.

West, J. B., Boyer, S. J., Graber, D. J., Hackett, P. H., Maret, K. H., Milledge, J. S., Peters, R. M., Jr, Pizzo, C. J., Samaja, M., Sarnquist, F. H. et al. (1983). Maximal exercise at extreme altitudes on Mount Everest. *J. Appl. Physiol.* **55**, 688-698.

Willems, N. J. and Armitage, K. B. (1975). Thermoregulation and water requirements in semiarid and montane populations of the least chipmunk, *Eutamias minimus*. I. Metabolic rate and body temperature. *Comp. Biochem. Physiol.* **51A**, 717-722.

Wunder, B. A. (1970). Energetics of running activity in Merriam's chipmunk, *Eutamias merriami*. *Comp. Biochem. Physiol.* **33**, 821-836.