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Maximal aerobic performance of deer mice in combined cold and exercise challenges

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Abstract In nature, animals frequently need to deal with several physiological challenges simultaneously. We examined thermoregulatory performance (body temperature stability) and maximal oxygen consumption of deer mice (*Peromyscus maniculatus*) during intense exercise at room temperature, acute cold exposure, and exercise during cold exposure. Results with exercise and cold exposure alone were consistent with previous studies: there was little difference between maximal metabolism elicited by exercise alone or cold exposure alone in warm-acclimated mice; after cold acclimation (9 weeks at 5 °C), maximal exercise metabolism did not change but maximum thermogenic capacity increased by >60%. Warm acclimated animals did not increase maximal oxygen consumption when exercise was combined with moderate cold (0 °C) and had decreased maximal oxygen consumption when exercise was combined with severe cold (−16 °C). Combined cold and exercise also decreased thermoregulatory performance and exercise endurance time. Cold acclimation improved thermoregulatory performance in combined cold and exercise, and there was also a slight increase in endurance. However, as for warm-acclimated animals, maximal exercise metabolism did not increase at low temperatures. We interpret these results as an indication of competition between thermoregulatory and locomotor effectors (brown adipose tissue and skeletal muscle) under the combined challenges of cold exposure and maximal exercise, with priority given to the locomotor function.

Keywords Metabolism · Exercise · Cold · Thermogenesis · Deer mouse

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Abbreviations *BAT* brown adipose tissue · T_b body temperature · $\dot{V}O_2$ rate of oxygen consumption · $\dot{V}O_2max$ maximal $\dot{V}O_2$ in exercise · $\dot{V}O_2sum$ maximal $\dot{V}O_2$ during cold exposure

Introduction

One of the central tenets of ecological and evolutionary physiology is that animal performance traits evolved under natural selection in response to challenges imposed by the environment, and numerous studies have explored the limits to performance in extremes of temperature, exercise, salinity, water stress, nutrient availability or quality, etc. In the interest of experimental simplicity, physiologists have usually examined performance limits individually (i.e., one stressor at a time). That approach has had great success, but in nature animals often must deal with multiple environmental challenges simultaneously, or undergo different activities in combination (e.g., Hammond and Kristan 2000; Kristan and Hammond 2000; Bennett and Hicks 2001). One example, especially relevant for small endotherms living at high latitudes or altitudes, is the combined demands of thermostatic heat production and locomotor exercise. Each of these elicits large increases in oxidative metabolism by itself, but at low environmental temperatures, demands for both occur simultaneously. In a mechanistic context, this is an interesting combination because it entails both overlapping and independent effectors and support systems. In many small eutherian mammals (especially after cold acclimation) a primary source of metabolic heat, brown adipose tissue (BAT), is dedicated exclusively to thermogenesis and in that sense can function independently of other effectors. However, the other main heat source for regulatory thermogenesis—skeletal muscles—must be 'shared' between locomotor and thermogenic needs, as must the central organs (cardiopulmonary system, etc.) supplying the effectors with oxygen and fuel.

As a further complication, heat produced as a byproduct of exercise may or may not be available to

supplement 'normal' thermogenesis (shivering or BAT activity). Although the question has not been extensively researched, it appears that at submaximal activity levels and moderate cold exposure, exercise heat production usually does not substitute for thermogenic requirements in small mammals (i.e., these requirements are additive, presumably because exercise disrupts pelage insulation and increases thermal conductance; Wunder 1970; Hart 1971). However, exercise heat is partially or completely substitutive in larger mammals (Taylor 1974) and small birds during both terrestrial exercise (Pohl and West 1973; Paladino and King 1984) and in flight and foraging activity (Webster and Weathers 1990).

Although thermogenic and locomotor costs seem to be additive during submaximal exercise in many small mammals, it is not clear how these two demands interact during maximal exercise—how does the presence of cold stress impact maximal aerobic capacity in exercise, does a simultaneous requirement for intense exercise affect thermogenesis during cold exposure, and how effectively is body temperature maintained under these conditions? These questions are likely to be ecologically relevant for winter-active small mammals, which have to move considerable distances at low ambient temperatures (T_as). There are several possible outcomes of combined maximal exercise and cold:

1. Additive costs, increased aerobic scope; i.e., maximal power output is higher in combined thermogenesis and exercise than in either activity alone; this presumes that effectors (skeletal muscle, BAT) are limiting but independent of each other and that central supply systems have sufficient capacity to handle both demands simultaneously.
2. Central constraint, fixed aerobic scope; i.e., power output by effectors is limited by nutrient or oxygen delivery from central supply systems, so maximal power output is unchanged when exercise and cold exposure are combined.
3. Functional competition within or among effectors; i.e., shivering may be incompatible with effective force generation for locomotion in skeletal muscles, or BAT thermogenesis cannot occur simultaneously with intense exercise, even if central supply systems are not limiting.

Under scenario 1 there would be no reduction in exercise capacity or thermoregulatory ability when both challenges are combined, but in scenarios 2 and 3, either exercise performance (maximum sustainable speed, endurance), thermoregulatory performance (stability of body temperature), or both would be reduced in combined cold and maximal exercise—a performance tradeoff. Of course, intermediate responses (e.g., a modest increase in maximal power output but decreased endurance and thermoregulatory performance) are possible.

We examined these predictions using a small rodent, the deer mouse (*Peromyscus maniculatus*). Deer mice are widely distributed in North America, frequently encounter low winter temperatures in many parts of their large range, and remain active throughout the year. In cold conditions they may use daily torpor as an energy-saving mechanism (e.g., Lynch et al. 1980; Tannenbaum and Pivorun 1988; Nestler 1990; Geiser 1991), but this does not preclude daily exposure to low T_a during the activity phase of the torpor cycle. Aerobic performance in deer mice has been intensively studied. Much is known about changes in aerobic capacity during exercise and thermogenesis in relation to temperature acclimation or acclimatization (Chappell 1984; Hayes and Chappell 1986, 1990; Hayes 1989a, 1989b), and considerable work has focused on adaptations to altitude in *P. maniculatus*, which is found from below sea level to above 4,000 m (Chappell and Snyder 1984; Chappell et al. 1988). These data point to the importance of combined exercise capacity and cold tolerance for deer mice in their natural habitats. Biophysical models in combination with thermoregulatory data suggest that deer mice experience winter temperatures that severely constrain exercise capacity (if exercise and thermostatic costs are additive). Furthermore, evidence from survivorship studies (Hayes and O'Connor 1999) suggests that high aerobic capacity in cold confers a fitness advantage in wild mice.

Materials and methods

Animals

We used a western subspecies of deer mice (*P. m. sonoriensis*) from a laboratory colony at the University of California, Riverside. The mice were third to fourth-generation descendents from 35 individuals captured in the White Mountains of eastern California. The breeding program was managed to maximize outcrossing and there was no intentional selection, except that the founding population was serologically tested to insure that none carried Sin Nombre virus (a variant of Hantavirus). During our tests, animals were maintained individually in standard mouse cages at room temperature (20–22 °C; the lower critical temperature of deer mice is 25–30 °C; Chappell 1985) with a photoperiod of 14L:10D. They were provided with bedding (wood shavings and cotton) and ad libitum water and rodent chow.

To determine the effects of cold acclimation on performance, we transferred mice into an environmental room maintained at 5 ± 0.5 °C for 9 weeks. The thermogenic capacity of deer mice acclimatizes to 5 °C within 5–6 weeks (E. Rezende, unpublished data). Aside from the temperature reduction during cold acclimation, all aspects of animal housing and care were constant throughout the study.

Oxygen consumption

We determined rates of oxygen consumption ($\dot{V}O_2$) with open-flow respirometry. Changes in O_2 concentration were measured with an Applied Electrochemistry S-3A analyzer and recorded on a Macintosh computer equipped with a National Instruments A-D converter and custom software ("LabHelper", www.warthog.ucr.edu). Gas flow was regulated with Tylan mass flow controllers upstream from the metabolism chambers; we used flow rates that maintained O_2 concentrations above 20.4% in all measurement conditions.

About 100 ml/min of sample gas was scrubbed of CO_2 and water vapor (soda lime and drierite) and routed through the oxygen sensor. We calculated $\dot{V}O_2$ as:

$$\dot{V}O_2 \text{ (in ml/min)} = \dot{V} \cdot (F_iO_2 - F_eO_2)/(1 - F_iO_2) \quad (1)$$

Where $\dot{V}$ is flow rate (ml/min STP) and $F_iO_2$and F_eO_2 are the fractional O_2 concentrations in incurrent and excurrent gas, respectively (F_iO_2 was 0.2095). The maximum cumulative error in $\dot{V}O_2$ calculations was $<4\%$ of measured values (based on the resolution of the S-3A relative to the change in O_2 concentration during tests, and the estimated calibration errors of the mass flow controller). The relative error between measurements was smaller because the repeatability of flow controller output was higher than the absolute accuracy of $\pm 2\%$.

Aerobic capacity in exercise

Maximum $\dot{V}O_2$ during exercise ($\dot{V}O_2$max) was determined by running mice in an enclosed motorized treadmill (Chappell 1984; Chappell and Snyder 1984; Hayes and Chappell 1990). The treadmill's working section was 6 cm wide, 7 cm high, and 13.5 cm long; the total enclosed gas volume was about 970 ml. We used a flow rate of 2100 ml/min STP of dry air. We tested each mouse's exercise performance in three thermal conditions: 18–21 °C (termed 'room temperature' in the rest of this paper), 0 °C to −4 °C (termed '0 °C'), and −14 °C to −18 °C (termed '−16 °C'). Low T_as were not maintained precisely since we did not want to leave mice in a cold treadmill long enough for temperature to reach steady-state. Mice experienced the three thermal conditions in random order.

To begin a test, we placed a mouse in the treadmill's working section and allowed a 1–1.5-min adjustment period before starting the tread at low speed (approximately 0.15 m/s). We increased speed in increments of 0.1 m/s every 30 s until the mouse could no longer maintain position and $\dot{V}O_2$ did not increase with increasing speed, at which time the tread was stopped. After the end of exercise, we continued to monitor metabolism for 1 min (when $\dot{V}O_2$ had begun to decrease), and then removed the animal. Tests lasted 4–10 min (2–6 min of tread movement). Reference readings of incurrent gas were obtained before and after each run. For most tests we obtained an index of thermoregulatory performance by measuring body temperature (T_b) immediately after removing the mouse from the chamber, using a rectal thermocouple connected to a Bailey BAT-12 thermometer (data obtained more than 30 s after opening the treadmill were discarded). A numerical score for run quality was assigned (Table 1) as a behavioral index of exercise performance.

Because of the relatively large volume of the treadmill and the short duration of exercise tests, we used the 'instantaneous' correction (Bartholomew et al. 1981) to compensate for mixing and resolve short-term changes. Effective volume of the treadmill, calculated from washout curves, was 903 ml. We applied the 'instantaneous' correction to O_2 concentration data, used Eqn 1 to calculate $\dot{V}O_2$, and then computed $\dot{V}O_2$max as the highest instantaneous $\dot{V}O_2$ averaged over continuous 1-min intervals (Chappell and Snyder 1984; Chappell et al. 1988).

Table 1 Scoring criteria for the 0–5 run quality scale

Score	Performance and behavior
0	No attempt to run; rapid apparent exhaustion
1	Some attempt to run but animal did not maintain position
2	Mouse maintained forward motion but often stopped and turned
3	Mouse maintained forward motion but had frequent, sustained contact with back wall of treadmill
4	Mouse ran strongly; occasionally contacted back wall of treadmill
5	Mouse ran continuously and attained speeds >0.6 m/s

Thermogenic capacity

Maximal thermogenic $\dot{V}O_2$, or 'summit' metabolism ($\dot{V}O_2$sum), was measured at moderately low temperatures in heliox (21% O_2, 79% He). Heat loss rates are several times higher in helox than in air (Rosenmann and Morrison 1974), and we could quickly elicit $\dot{V}O_2$sum at T_as between 0 °C and −10 °C, depending on the size and individual thermogenic capacity of the mouse. Use of these relatively warm temperatures minimized the risk of cold injury (no animal was frostbitten during our study).

The 460-ml Lucite metabolic chamber contained a small amount of wood shavings and was flushed with heliox at a flow rate of 1700 ml/min STP. We started runs at T_as of 0 °C to −3 °C and monitored $\dot{V}O_2$ as T_a dropped at about 0.5–1 °C/min. The S-3A gives unstable results with mixtures of air and heliox (personal observations), and the first 30–60 s of data after sealing the mouse in the chamber were unusable (subsequently the instrument stabilized after residual N_2 was flushed from the system). When $\dot{V}O_2$ began to decline, or if it remained constant over a 3 °C drop in T_a, we terminated the test and returned the mouse to its cage. Measurements lasted 6–12 min. As for exercise $\dot{V}O_2$max, we took reference readings before and after measurements, calculated $\dot{V}O_2$ from Eqn. 1, and computed $\dot{V}O_2$sum as the highest $\dot{V}O_2$ averaged over continuous 1-min intervals (use of the instantaneous correction was unnecessary because of the high ratio of flow rate to chamber volume). We measured T_b immediately after removing the mouse from the chamber, as described for treadmill tests.

Analysis and statistics

We used covariance analysis (ANCOVA, with mass as covariate) to test for differences among categorical variables (sex, test condition), and paired *t*-tests to compare performance of individuals before and after cold acclimation. Post-hoc Scheffe tests were used to test for differences among ANCOVA groups. We compared nonparametic run quality scores with Kruskall-Wallace and Mann-Whitney U tests, and used Kendall's tau to look for correlations between run quality and other variables. Data are shown as mean ± SD. The critical significance level α was 0.05; we used a sequential Bonferroni correction to adjust α in multiple simultaneous tests to avoid Type I errors (Rice 1989). Analyses were performed using Statistica software for the Macintosh (Statsoft, USA) and a custom-written Bonferroni program.

Results

We obtained data from 48 warm-acclimated deer mice (25 males, 23 females); 15 of these animals (7 males, 8 females) were also tested after cold acclimation. All mice appeared healthy throughout experiments, and none were obese. The mean mass of cold-acclimated mice (23.4 ± 2.7 g) did not differ from their mass when warm-acclimated (24.4 ± 3.7 g; $P = 0.48$), or from the mean mass of all 48 warm-acclimated animals (24.0 ± 4.1 g; $P = 0.32$). Some of the warm-acclimated individuals were not tested in all of the four experimental protocols, but all cold-acclimated mice were tested in all four protocols before and after acclimation.

As expected, mass was positively correlated with both $\dot{V}O_2$max and $\dot{V}O_2$sum, but due to the relatively small

mass range in tested mice (16–36 g), only exercise $\dot{V}O_2max$ at −16 °C was significantly correlated with mass in both warm- and cold-acclimated animals ($r = 0.29$, $P = 0.046$ and $r = 0.597$, $P = 0.019$, respectively). T_b was not correlated with mass in any test condition in warm-acclimated mice, but was significantly positively correlated with mass in cold-acclimated mice in exercise at 0 °C and in heliox cold exposure tests ($r = 0.669$, $P = 0.0060$ and $r = 0.658$, $P = 0.0077$, respectively). There were no differences between males and females in any test or acclimation state for either metabolism or T_b.

Warm-acclimated mice

Maximal $\dot{V}O_2$ did not differ when measured in room temperature exercise, in exercise at 0 °C, or in heliox cold exposure, but was significantly lower in exercise at −16 °C (Fig. 1a; Table 2). T_b did not differ between room temperature and 0 °C exercise, but was significantly lower in exercise at −16 °C and heliox cold exposure tests (Fig. 1b; Table 3). The approximate rate of change in T_b (estimated from running duration and the total change in T_b from a normothermic T_b of 38 °C; we did not measure each individual's pre-run T_b in order to minimize disturbance) was a decrease of 0.27 °C/min in 0 °C exercise and a decrease of 1.31 °C/min in −16 °C exercise; during room temperature exercise, T_b increased at approximately 0.16 °C/min. The duration of running during exercise tests was maximal at room temperature and declined significantly when animals were run at low temperatures (Fig. 2; ANCOVA with mass as covariate; $F_{2,58} = 15.2$, $P < 0.0001$). Running duration differed significantly between room temperature and 0 °C and room temperature and −16 °C (Sheffe tests, $P < 0.0047$), but there were no duration differences between 0 °C and −16 °C (Sheffe test, $P = 0.189$).

The running quality index (Table 1; Fig. 3) declined significantly as treadmill temperature decreased ($P = 0.016$, Kruskal-Wallace test; mean quality scores were 3.2 ± 1.6 in room temperature tests, 2.2 ± 1.2 at

Table 2 Differences in maximal oxygen consumption in different test conditions. The table shows *P* values from separate Sheffe tests for warm-acclimated mice (*upper right*) and cold-acclimated mice (*lower left*)

	Exercise, 20 °C	Exercise, 0 °C	Exercise, −16 °C	Heliox
Exercise, 20 °C	-	0.99	< 0.0001	0.97
Exercise, 0 °C	0.78	-	< 0.0001	0.97
Exercise, −16 °C	0.99	0.83	-	< 0.0001
Heliox	< .0001	< .0001	< .0001	-

Table 3 Differences in body temperature in different test conditions. The table shows *P* values from separate Sheffe tests for warm-acclimated mice (*upper right*) and cold-acclimated mice (*lower left*)

	Exercise, 20 °C	Exercise, 0 °C	Exercise, −16 °C	Heliox
Exercise, 20°C	-	0.11	< 0.0001	< 0.0001
Exercise, 0°C	0.76	-	0.0013	< 0.0001
Exercise, −16°C	0.00081	0.0072	-	0.000193
Heliox	< 0.0001	< 0.0001	0.0047	-

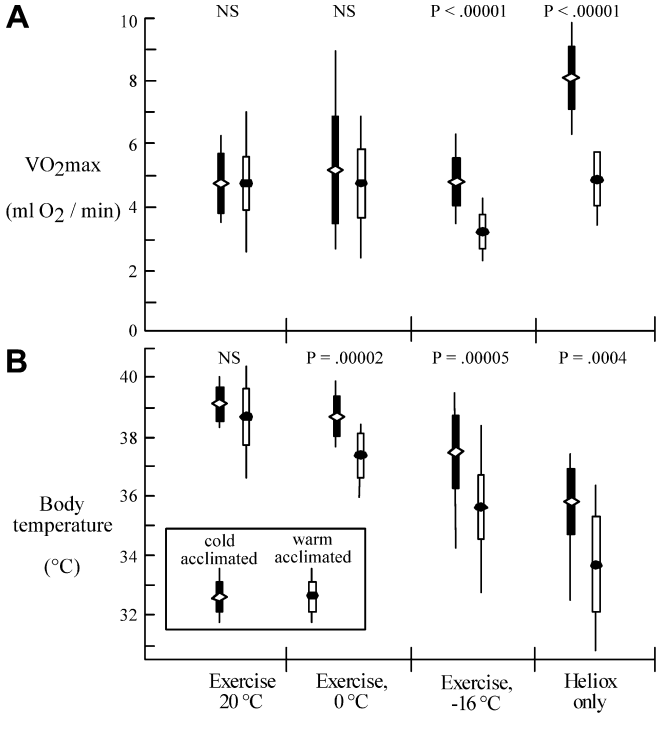

Fig. 1A, B Maximum oxygen consumption ($\dot{V}O_2max$; **A**) and body temperature (**B**) of deer mice in exercise at three ambient temperatures, and in acute cold exposure in a helium-oxygen atmosphere (heliox). *Vertical lines* are ranges, *boxes* are standard deviations, and *ovals* and *diamonds* are means. Data are shown for warm-acclimated mice (open boxes; $N = 48$) and cold-acclimated mice (*solid boxes*; $N = 15$), with *P*-values for differences between the two acclimation states

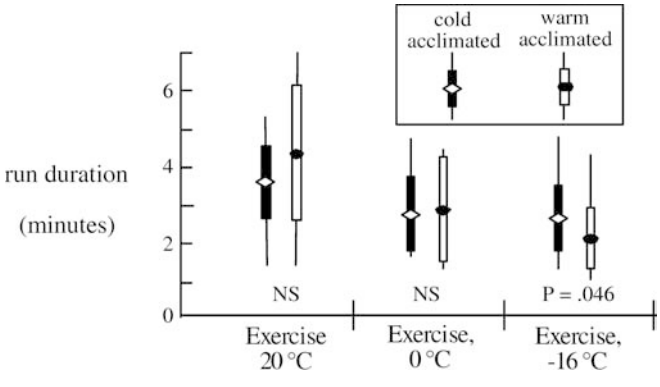

Fig. 2 The duration of exercise tests in our standard protocol at three ambient temperatures. Tests were terminated when mice could no longer maintain position in the treadmill. Sample sizes and symbols as in Fig. 1

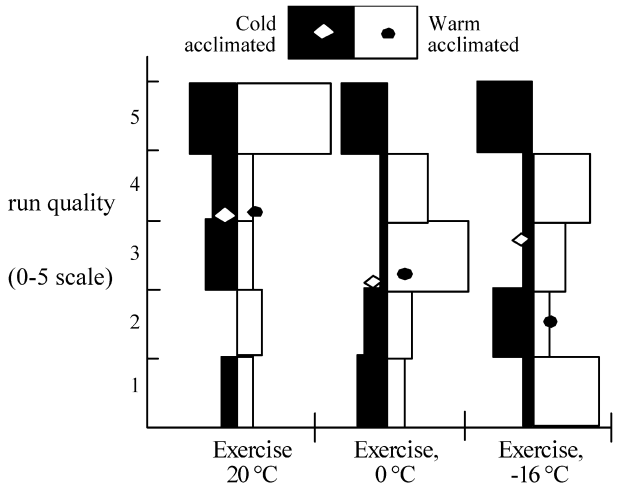

Fig. 3 Histogram of subjective running quality (Table 1) during our standard exercise protocol at three ambient temperatures for warm-acclimated and cold-acclimated deer mice (*open bars* and *dark bars*, respectively). The *length of the bars* is proportional to the number of samples in each run quality category; means are indicated with *diamonds* or *ovals*; total sample sizes as in Fig. 1

0 °C, and 1.6 ± 1.5 at −16 °C). Run quality was positively correlated to running duration at all three exercise temperatures (room temperature: Kendall's tau = 0.70, $z = 4.45$, $P < 0.0001$; 0 °C: tau = 0.51, $z = 3.17$, $P = 0.00153$; −16 °C: tau = 0.48, $z = 3.05$, $P = .00227$). In both room temperature and severe cold (−16 °C), the duration of running was positively correlated with post-run T_b ($F_{1,12} = 11.2$, $r^2 = .48$, $P = 0.0059$ and $F_{1,19} = 6.5$, $r^2 = 0.26$, $P = 0.019$, respectively), but duration was not correlated with T_b in tests at 0 °C. Neither running quality nor duration was significantly related to $\dot{V}O_2$max ($P > 0.1$).

Cold-acclimated mice

There was no difference in exercise $\dot{V}O_2$max at any temperature (Sheffe test; $P > 0.75$), but $\dot{V}O_2$sum (heliox cold exposure) averaged 64% higher than $\dot{V}O_2$max during exercise (8.09 ± 0.99 ml O_2/min versus 4.93 ± 1.24 ml O_2/min, respectively; $P < 0.0001$). As for warm-acclimated mice, T_b was strongly affected by test conditions, being highest for room-temperature exercise and lowest in the heliox cold exposure test (Fig. 1b; Table 2). The approximate rate of decrease in T_b (estimated as described for warm-acclimated animals) was 0.30 °C/min during exercise at 0 °C and 0.36 °C/min during exercise at −16 °C; during room-temperature exercise T_b increased at approximately 0.36 °C/min. Running duration during exercise tests declined slightly with decreasing T_a (Fig. 2; ANCOVA with mass as covariate; $F_{2,41} = 3.34$, $P = 0.045$).

Running quality did not differ among the three test temperatures (Fig. 3; $P = 0.91$, Kruskal-Wallace test), with an average quality score of 2.7 ± 1.7. However, running quality was positively correlated to the duration of running under all three exercise conditions (room temperature: Kendall's tau = 0.62, $z = 3.2$, $P = 0.00134$; 0 °C: tau = 0.66, $z = 3.45$, $P = 0.000555$; −16 °C: tau = .78, z = 4.04, P < .0001). Running quality predicted $\dot{V}O_2$max for exercise at −16 °C ($F_{1,13} = 5.26$, $P = 0.0392$), but not at other temperatures ($P > 0.2$). Run duration was not significantly correlated with either $\dot{V}O_2$max ($P > 0.1$) or post-run T_b ($P > 0.06$) in any test condition.

Changes resulting from cold acclimation

Compared to warm-acclimated mice, cold-acclimated animals had a greatly elevated $\dot{V}O_2$sum in the heliox test (Fig. 1a; 8.09 ± 0.99 ml O_2/min versus 4.91 ± 0.85 ml O_2/min, respectively; $P < 0.0001$). Acclimation regime did not affect exercise $\dot{V}O_2$max at room temperature or 0 °C ($P = 0.99$; $P = 0.20$, respectively), but cold-acclimated mice had substantially higher $\dot{V}O_2$max during exercise at −16 °C than warm-acclimated animals (4.81 ± 0.83 versus 3.19 ± 0.52 ml O_2/min, respectively; $P < 0.0001$).

Besides having higher metabolic power output in severe cold (−16 °C exercise and especially heliox cold exposure), cold-acclimated mice were able to maintain higher T_bs than warm-acclimated mice in most test conditions (Fig. 1b). There was no difference in T_b in room-temperature exercise ($P = 0.17$), but T_b in cold-acclimated mice averaged 1–2 °C higher than that of warm-acclimated mice in low-temperature exercise and heliox cold-exposure tests (0 °C exercise: 38.8 ± 0.7 °C versus 37.4 ± 0.85 °C; −16 °C exercise: 37.3 ± 1.6 °C versus 35.3 ± 2.6 °C; heliox cold exposure: 35.9 ± 1.2 °C versus 33.8 ± 1.5 °C; $P < 0.0007$ in all cases). Estimated rates of change of T_b during exercise were significantly different at all three temperatures ($t = -2.42$, $P = 0.024$ at room temperature, $t = -5.15$, $P < 0.0001$ at 0 °C, and $t = -2.65$, $P = 0.012$ at −16 °C).

There were only two differences between warm- and cold-acclimated animals in the duration or subjective quality of exercise, both at −16 °C (Fig. 2). Cold-acclimated animals had a significantly higher run quality score (2.7 ± 1.8 versus 1.6 ± 1.5; $P = 0.043$, Mann-Whitney U test) and were able to continue treadmill exercise for slightly longer than warm-acclimated mice.

Discussion

Aerobic capacity in cold or exercise alone

In general, our results for maximal aerobic capacity during exercise at room temperature ($\dot{V}O_2$max), and for thermogenic heat production in the absence of forced exercise ($\dot{V}O_2$sum), are similar to data from previous studies of deer mice, other *Peromyscus* species, and many small mammals (e.g., Wickler 1980; Chappell 1984; Chappell and Snyder 1984; Hayes and Chappell 1986; Hinds and Rice-Warner 1992; Hinds et al. 1993;

Chappell et al. 2003). Locomotor performance during our treadmill tests varied considerably between individuals, but it is noteworthy that we did not find correlations between the animals' ability or motivation to run—as coded in the numeric run quality score—and $\dot{V}O_2$max. The absence of such a correlation is evidence that the treadmill protocol was a reliable measure of maximal aerobic capacity during generalized muscular exercise, and was not significantly biased by behavioral or motivational differences among the mice (we would have expected a positive correlation between run quality scores and $\dot{V}O_2$max if the latter were true). There was no difference between $\dot{V}O_2$max and $\dot{V}O_2$sum in our warm acclimated animals (similar to the findings of Chappell 1984); other studies have often found slightly higher $\dot{V}O_2$sum than $\dot{V}O_2$max (Segrem and Hart 1967; Hayes and Chappell 1986; Chappell and Bachman 1994; but see Seeherman et al. 1981 and Hinds and Rice-Warner 1992).

As expected, cold acclimation dramatically increased thermogenic capacity in acute cold exposure (e.g., Rosenmann et al. 1975; Heimer and Morrison 1978; Wickler 1980; Hayes and Chappell 1986). We found a 65% increase in $\dot{V}O_2$sum after cold acclimation, which is a larger change than reported by other laboratory studies of cold acclimation in deer mice (e.g., 31%; Hayes and Chappell 1986) but comparable to the increase in thermogenic $\dot{V}O_2$max of winter-acclimatized wild deer mice in California (about 50%; Hayes 1989a, 1989b) and of winter-acclimatized *Peromyscus leucopus* in Michigan (about 70%; Wickler 1980). Presumably the increased thermogenic capacity after cold acclimation was largely a result of upregulated BAT (e.g., Heldmaier and Buchberger 1985; Himms-Hagen 1986, 1990).

Combined exercise and cold

Previous work on the effects of T_a on energy costs of submaximal locomotion suggest that thermogenic and exercise costs are often additive in small mammals (Wunder 1970; Hart 1971): in other words, at a constant exercise intensity, metabolic rate increases when T_a decreases. In warm-acclimated deer mice, our results for combined exercise and varying degrees of cold exposure suggest that locomotor and thermogenic costs are not additive at maximal exercise intensities: there was no increase in $\dot{V}O_2$max as temperature decreased (in fact, $\dot{V}O_2$max was significantly reduced in tests at −16 °C), and T_b tended to decline during exercise tests at low T_a (next paragraph). Those findings are consistent with either a central supply system limitation to aerobic scope (i.e., insufficient pulmonary oxygen uptake or cardiovascular transport to support both exercise and thermogenesis) or within-effector function competition (i.e., skeletal muscles cannot be used simultaneously for shivering and locomotion). Cold exposure alone (in heliox) elicited essentially the same maximal $\dot{V}O_2$ as occurred during exercise. Taken together, the exercise and heliox data are most consistent with a central limitation of aerobic capacity, but do not provide a strong test of the effector competition hypotheses, since warm-acclimated animals have a relatively small capacity for nonshivering thermogenesis in BAT.

Since heat production during maximal exercise did not increase as T_a declined, thermoregulatory performance (ability to maintain normal T_b) should suffer unless exercising mice can substantially decrease conductance at very low T_a. That seems unlikely, as the lower critical temperature in resting *P. maniculatus* is >20 °C (Chappell and Holsclaw 1984; Chappell 1985) and locomotor activity apparently disrupts pelage and increases conductance in small mammals (Wunder 1970). Hence, our findings of low T_b compared to normothermic values (37–38 °C in resting deer mice at 20 °C; Chappell 1985) at the conclusion of low-temperature exercise tests are not surprising. It was also unremarkable to find declines in exercise performance (in both the subjective running quality score and in the duration of effective running in our standard treadmill protocol; Figs. 2, 3) when mice were simultaneously challenged with low T_a (the associated decline in T_b may have contributed to reduced endurance).

Our results for combined exercise and cold exposure in cold-acclimated deer mice were surprisingly similar to those for warm-acclimated mice (Figs. 1, 2). As for warm-acclimated animals, there was no evidence for additivity of thermogenic and exercise costs at maximal exercise intensities, despite the fact that cold-acclimated deer mice have greatly enhanced thermogenic $\dot{V}O_2$ when not exercising (Fig. 1; Hayes and Chappell 1986). Since BAT and skeletal muscle can function independently and simultaneously—unlike the inherent competition between shivering and locomotion in skeletal muscle—our expectation was for a substantial elevation of $\dot{V}O_2$max in low temperature exercise tests (compared to $\dot{V}O_2$max in room temperature exercise) after cold acclimation. That did not occur, but cold-acclimated animals did have improved thermoregulatory performance at 0 °C and −16 °C (significantly lower rates of change in T_b, and significantly higher post-test T_b; Fig. 2) compared to warm-acclimated mice. Cold-acclimated mice also had significantly greater $\dot{V}O_2$max during −16 °C exercise than warm-acclimated mice, perhaps because of higher T_b, and their running quality was not reduced at low T_a.

Despite the presence of substantial aerobic capacity in each of two effectors (BAT and muscle) after cold acclimation, there was no increase in exercise $\dot{V}O_2$max at low temperatures. Superficially these findings provide additional support for a central limitation model, but that conclusion—at least in its simplest form—is contradicted by the results of heliox tests in cold-acclimated mice: their $\dot{V}O_2$sum was 65% greater than the $\dot{V}O_2$max at any temperature, indicating that central supply systems are capable of supporting considerably more metabolic power output (at least in terms of oxygen delivery) than occurred during any tested combination of cold and exercise. Our findings could be an

experimental artifact of the stress of forced exercise, but this seems unlikely given that forced exercise in rats does not inhibit BAT thermogenesis in response to noradrenaline (Shibata and Nagasaka 1987; Wickler et al. 1987; but see Richard et al. 1992; Ardeval et al. 1996). Accordingly, the most likely explanation for constant exercise $\dot{V}O_2$max across a range of temperatures is competition between effectors, although central supply limitations may also apply. We speculate that constraints to cardiac output require that blood be routed away from BAT during intense locomotor exercise in order to maintain adequate perfusion in the large volume of skeletal muscle (i.e., priority is given to skeletal muscle function). That strategy seems reasonable in many ecological contexts (e.g., escaping from predators should have more fitness value than careful maintenance of T_b). Since BAT is a highly aerobic tissue, vasoconstriction would suppress its metabolism during exercise. During 'pure' cold exposure in the absence of locomotion, the relatively small volume of BAT can be richly perfused (e.g., Wickler and Horowitz 1984) and hence can generate maximal heating power, in combination with some shivering thermogenesis (in terms of heat production, the power density of BAT is high relative to that of other tissues; e.g., Foster 1986).

How relevant are these findings for deer mice in natural conditions? The source population for our laboratory colony was the White Mountains in eastern California, with local altitude around the collection site ranging from 3,500 m to 4,000 m. Winter snow surface temperatures there are normally well below 0 °C, and even in summer, nighttime temperatures are often below 5 °C (M.A. Chappell, unpublished data; Hayes 1989). Live-trapping data show that wild deer mice in the White Mountains routinely travel long distances within extensive home ranges (nightly movement over linear distances of hundreds of meters occurs regularly; J.P. Hayes, personal communication) and voluntary wheel-running in captive animals can exceed 15 km/night at velocities close to the maximum aerobic speed of 0.6–1 m/s (M.A. Chappell, unpublished data). The running velocities preferred by free-living deer mice are unknown but some small mammals normally travel at or near their maximum aerobic speed (Kenagy and Hoyt 1989), as these speeds provide the most efficient cost of transport (and for travel through open areas, high speed minimizes exposure to predators). Thus, high-speed running in cold conditions is likely a common behavior in deer mice. Our data suggest that such activity may compromise thermoregulation, but wild individuals probably do not run for the extended periods (several minutes) used in our exercise tests. Given the thermal characteristics of deer mice, use of fairly brief bouts of maximal exercise (< 1 min) would not engender large drops in T_b, even in severe cold. However, the lack of additivity of thermal and exercise costs, and the resulting trade-off of locomotor and thermoregulatory performance, may preclude deer mice from long periods of intense activity in winter conditions.

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