Voluntary running in deer mice: speed, distance, energy costs and temperature effects

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Summary

The energetics of terrestrial locomotion are of considerable interest to ecologists and physiologists, but nearly all of our current knowledge comes from animals undergoing forced exercise. To explore patterns of energy use and behavior during voluntary exercise, we developed methods allowing nearly continuous measurements of metabolic rates in freely behaving small mammals, with high temporal resolution over periods of several days. We used this approach to examine relationships between ambient temperature (T_a), locomotor behavior and energy costs in the deer mouse, a small mammal that routinely encounters a large range of temperatures in its natural habitat. We tested for individual consistency in running behavior and metabolic traits, and determined how locomotor costs vary with speed and T_a. Because of the importance of thermoregulatory costs in small mammals, we checked for substitution of exercise heat for thermostatic heat production at T_a below the thermal neutral zone and determined the fraction of the daily energy budget comprising exercise costs.

Locomotor behavior was highly variable among individuals but had high repeatability, at least over short intervals. We found few temperature-related changes in speed or distance run, but T_a strongly affected energy costs. Partial substitution of exercise heat for thermogenic heat occurred at low T_a. This reduced energy expenditure during low-temperature running by 23–37%, but running costs comprised a fairly minor fraction of the energy budget, so the daily energy savings *via* substitution were much smaller. Deer mice did not adjust running speed to maximize metabolic economy, as they seldom used the high speeds that provide the lowest cost of transport. The highest voluntary speeds (4–5 km h^{-1}) were almost always below the predicted maximal aerobic speed, and were much less than the species' maximal sprint speed. Maximum voluntarily attained rates of oxygen consumption ($\dot{V}_{O_2}$) were highest at low T_a, but rarely approached maximal $\dot{V}_{O_2}$ during forced treadmill exercise. Mean respiratory exchange ratios coincident with maximal voluntary $\dot{V}_{O_2}$ increased slightly as T_a declined, but were always below 1.0 (another indication that metabolic rate was less than the aerobic maximum). Individuals with high running performance (cumulative distance and running time) had high resting metabolism, which suggests a cost of having high capacity or propensity for activity.

Key words: aerobic capacity, deer mouse, exercise, locomotion, maximal oxygen consumption, metabolism, *Peromyscus maniculatus*, wheel-running.

Introduction

Over the past three decades, exercise and environmental physiologists have painstakingly described the energetics of locomotion in a wide range of animals. In quadrupedal mammals running at near-thermoneutral temperatures, the relationship between metabolic rate and running speed is approximately linear over a broad range of speeds for most species (Taylor et al., 1970, 1982), with the slope of the regression of metabolism *vs* speed inversely related to body mass and the intercept (power output at zero speed) elevated above resting metabolism. Considerably less is known about locomotion energetics at low ambient temperatures, where energy costs of exercise may interact with the need for regulatory thermogenesis. In large mammals such as humans

(Brooks and Fahey, 1984), and in birds (e.g. Paladino and King, 1984; Webster and Weathers, 1990), heat produced as a byproduct of exercise can substitute for heat that would otherwise need to be produced by shivering or non-shivering thermogenesis to maintain body temperature in cold conditions. A few studies (Wunder, 1970; Hart, 1971) suggest that in small mammals, exercise and thermogenic costs are largely additive – that is, exercise heat cannot be substituted for thermogenesis, possibly because locomotor movements disrupt pelage insulation or affect peripheral circulation. Completely additive thermogenic and exercise costs could result in increasing constraints on the capacity for sustained exercise as temperature drops, unless maximal power output in

combined exercise and thermogenesis is higher than in exercise alone. That possibility has received little attention.

Another uncertainty about running energetics concerns the manner in which exercise costs are measured: except for studies on humans, nearly all data on the metabolic costs of running have been obtained from animals forced to run on treadmills, sometimes with the added complication of a face-mask for measurements of gas exchange. It is unclear if animals engaged in voluntary locomotion experience the same energy costs elicited by forced exercise. For example, data from horses (Hoyt and Taylor, 1981), ground squirrels (Hoyt and Kenagy, 1999; Kenagy and Hoyt, 1989), and a few other species (e.g. Pennycuick, 1975; Perry et al., 1988) show that mammals often prefer to travel within narrow speed ranges, apparently because of gait- and speed-related optima in locomotor efficiency (i.e. cost of transport, J km^{-1}) or biomechanical factors such as muscle and tendon stress (e.g. Wickler et al., 2001, 2003). Hence it is possible that small mammals preferentially use particular speed ranges that confer lower costs of transport than may be apparent in forced exercise protocols, or that the kinematics of locomotion or aspects of energy metabolism (such as substrate utilization) differ between forced and voluntary running, perhaps because of stress responses or other artifacts of forced exercise.

In this paper we use the North American deer mouse *Peromyscus maniculatus* to examine voluntary exercise across a range of ambient temperatures (T_a). We developed equipment and methods that provide nearly continuous long-duration records of energy metabolism and wheel-running performance in unrestrained, freely behaving small mammals, with high temporal resolution. To our knowledge, this is the first time such measurements have been accomplished. We used these data to examine relationships between resting metabolism, maximal aerobic capacity, T_a, exercise intensity and preferred speeds, and metabolic power use.

Deer mice are good natural models for studies of exercise physiology. Their thermal and aerobic physiology have been intensively studied and much is known about aerobic capacity changes in relation to temperature acclimation and acclimatization (Hayes and Chappell, 1986, 1990; Hayes, 1989a,b; Rezende et al., 2004) and adaptations to oxygen availability across a wide altitudinal range (below sea level to above 4000 m; Chappell and Snyder, 1984; Chappell et al., 1988). The species' maximal sprint-running speeds have also been measured (Djawdan and Garland, 1988). The primary questions we address here – (1) What is the energetic cost of voluntary locomotion? (2) How does it change with ambient temperature? (3) Are particular running speeds preferred? (4) How is voluntary running performance related to aerobic traits such as resting metabolism and maximal aerobic capacity? – are closely relevant to the ecology of deer mice. Field studies at a high-altitude site (Hayes and O'Connor, 1999; J. P. Hayes, personal communication) show that these mice routinely move across hundreds of meters of linear distance nightly. Moreover, in many parts of their extensive geographic range, deer mice seldom encounter thermoneutral temperatures (approximately 25–35°C; Chappell, 1985) during their above-ground nocturnal activity periods. Some populations from high altitudes must deal with activity temperatures that rarely if ever exceed 10°C, even in summer (Hayes, 1989a,b), and are often much colder in winter (M. A. Chappell, personal observations). Recent work shows that maximum metabolic power output during forced exercise does not increase at low ambient temperatures, even in cold-acclimated deer mice that have considerably elevated power output during maximal thermogenesis (Chappell and Hammond, 2003). Therefore, unless some substitution of exercise heat for thermogenic heat production occurs, the sustained locomotor capacity of these mice will be severely constrained at the low ambient temperatures that they routinely encounter in nature.

Materials and methods

Animals

We used a western subspecies of deer mice (*Peromyscus maniculatus sonoriensis* Wagner) from our captive colony at the University of California, Riverside (UCR; elevation 340 m). The mice were third- to sixth-generation descendents from 35 individuals collected in the White Mountains of eastern California (local elevation 3500–3900 m). Breeding was managed to maximize outcrossing and there was no intentional selection, except that the founding population was tested to insure that none carried Sin Nombre virus (O'Connor et al., 1997). Animals were housed in standard mouse cages (27.5 cm×17 cm×12 cm, L×W×H), without access to running wheels, at room temperature (22–24°C). They were provided with bedding (wood shavings and cotton) and water and rodent chow *ad libitum*.

Gas exchange measurements

For both treadmill tests and voluntary exercise measures, we used positive-pressure, flow-through respirometry to determine rates of oxygen consumption ($\dot{V}_{O_2}$); we also measured carbon dioxide production ($\dot{V}_{CO_2}$) during voluntary exercise. Oxygen concentration changes during treadmill tests were measured with an Applied Electrochemistry S-3A (Sunnyvale, CA, USA); for voluntary exercise measurements we used an 'Oxzilla' dual-channel O$_2$ analyzer (Sable Systems; Henderson, NV, USA) and two Sable Systems CA-2A CO$_2$ analyzers (one oxygen channel and one CO$_2$ analyzer for each of two mice measured simultaneously). We regulated air flow with upstream mass flow controllers [Applied Materials (Sunnyvale, CA, USA), Tylan (Billerica, MA, USA) or Porter Instruments (Hatfield, PA, USA)], using flow rates that maintained excurrent O$_2$ concentrations above 20.4%. About 100 ml min^{-1} of excurrent air was subsampled and analyzed for O$_2$ and CO$_2$. Data from gas analyzers and other instruments were recorded on Macintosh computers equipped with A-D converters (National Instruments, Austin, TX, USA) and 'Labhelper' software (Warthog Systems, warthog.ucr.edu).

Different conversion equations were used to compute $\dot{V}_{O_2}$

for treadmill tests and during voluntary exercise. For treadmill tests, we scrubbed subsampled air of water vapor and CO_2 (Drierite and soda lime, respectively) prior to gas analysis and calculated $\dot{V}_{O_2}$ as:

$$\dot{V}_{O_2} = \dot{V} \times (FI_{O_2} - FE_{O_2}) / (1 - FE_{O_2}) , \qquad (1)$$

where $\dot{V}_{O_2}$ is flow rate (ml min^{-1} STP; Standard Temperature and Pressure) and FI_{O_2} and FE_{O_2} are the fractional O_2 concentrations in incurrent and excurrent air, respectively (FI_{O_2} was 0.2095 and FE_{O_2} was always >0.204).
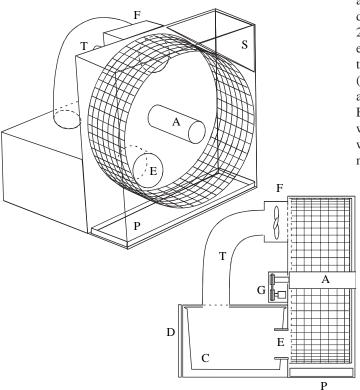
For measurements of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ during voluntary exercise, we dried subsampled air with magnesium perchlorate (Drierite® interacts with CO_2). We did not remove CO_2 as required for Equation 1 (in order to avoid the large volumes of soda lime or frequent scrubber changes that otherwise would be necessary for these long-duration tests) and calculated $\dot{V}_{O_2}$ as:

$$\dot{V}_{O_2} = \dot{V} \times (FI_{O_2} - FE_{O_2}) / [1 - FE_{O_2}(1 - RER)] , \qquad (2)$$

where RER is the respiratory quotient. Based on preliminary data and previous measurements on deer mice, we used an RER of 0.85 and calculated $\dot{V}_{CO_2}$ as:

$$\dot{V}_{CO_2} = \dot{V} \times (FE_{CO_2} - FI_{CO_2}) / \{1 - FE_{CO_2}[1 - (1/RER)]\} , \quad (3)$$

where FI_{CO_2} and FE_{CO_2} are the fractional incurrent and excurrent CO_2 concentrations (FI_{CO_2} was approximately 0.00037). We used the same RER (0.85) as in Equation 2. Note that if metabolic rate is changing rapidly, conversion equations that substitute measured gas concentrations for RER (e.g. use of CO_2 concentration to calculate $\dot{V}_{O_2}$) are potentially inaccurate unless there is close temporal synchronization of measurements of O_2 and CO_2 concentrations; such synchronization is difficult to achieve for rapid metabolic events if response times differ for O_2 and CO_2 analyzers. For this reason, we took a conservative approach and used equations based on constant RER. Values of RER between 0.7 and 1.0 had little effect on calculated $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. The maximum possible error introduced by use of a constant RER of 0.85 was 3% of calculated $\dot{V}_{O_2}$ and <0.2% of calculated $\dot{V}_{CO_2}$; actual errors (since RER was between 0.78 and 0.9) were 1.5% or less in $\dot{V}_{O_2}$ and <0.1% in $\dot{V}_{CO_2}$.

Respirometry during voluntary exercise

Like many small rodents, deer mice readily use running wheels, so to measure voluntary exercise performance we enclosed a commercially available rodent wheel (Lafayette Instruments, Lafayette, IN, USA; stainless steel and Plexiglas construction; circumference 1.12 m; Swallow et al., 1998) and a standard plastic mouse cage within an airtight Lucite® housing (Fig. 1; http://www.biology.ucr.edu/people/faculty/ Garland/Wheel_Metab_Alone_1.jpg; http://www.biology.ucr. edu/people/faculty/Garland/Wheel_Metab_Two_2.jpg). Mice could enter and exit the wheel at will through an access port (diameter 7.7 cm) cut into the side of the mouse cage. Wheel rotation turned a small generator, producing a voltage proportional to rotation speed and polarized to the direction of rotation. Paired incurrent and excurrent ports provided for air flow (2500 ml min^{-1}, ±1%), and an internal fan rapidly recirculated air within the enclosure to facilitate mixing. The mouse cage contained bedding (wood shavings), a food hopper and a drinking tube. Food and water were available *ad libitum* and mice were left in enclosures for periods of 48–96 h, with data recorded every 1.5 s. Computer-controlled valves took 2.5 min reference readings every 45 min. Two of the wheel enclosures were housed in a large incubator that controlled temperature (3, 10, or 25°C, ±0.5°C) and photoperiod (12 L:12 D, dark period = 19:00 h–07:00 h, which was approximately the same as the light cycle in our animal room). Every few days, we tested rotational resistance by spinning wheels to high speed (~80 r.p.m.) with an electric drill fitted with a rubber friction disk, and then monitoring the time needed for speed to decay to zero. No appreciable resistance changes occurred over the course of the study, nor did resistances differ between the two wheels.

Most unused volume in the enclosures was filled with plastic inserts (the four corners of the wheel housing) or high-density foam (the space surrounding the mouse cage;

Fig. 1. Schematic diagram of the running wheel enclosure. A, wheel axle (attached to the back side of the housing); F, fan; T, recirculation tube; C, mouse cage; D, access door; E, wheel entry tube; P, waste pan; G, wheel speed generator; S, space filler. Not shown: food hopper, drinking tube, air access ports, other space fillers in corners of wheel housing and around mouse cage. The internal volume (without cage, bedding, and food) is approximately 22.7 liters.

Fig. 1), and the remaining internal volume was about 22.6 liters after accounting for displacements of the wheel, cage, food and bedding. Even with mixing from the recirculating fan, this large volume resulted in a slow response to changes in gas concentration. Therefore, we used the 'instantaneous' transformation (Bartholomew et al., 1981) to provide accurate resolution of short-term metabolic changes. To determine the effective volume for this transformation, we flowed gas through the system at the standard rate of 2500 ml min^{-1}, established a stable baseline concentration, and then instantly switched to a different O_2 concentration at the same flow rate (we used air and a mixture of 14% O_2, 86% N_2) while recording the response to the step change. The time lag between gas switches and detection by the gas analyzers was approximately 20 s for CO_2 and 45 s for O_2, and effective volume was estimated as 17.0 liters.

At this combination of flow rate and effective volume, the instantaneous transformation is sensitive to small fluctuations in O_2 data (from electrical noise, air pressure transients, etc.). We used a very stable O_2 analyzer and each recorded datum was the average of several hundred readings during the 1.5 s inter-sample interval. Nevertheless, additional smoothing was necessary to obtain usable $\dot{V}_{O_2}$ records. Experimentation with step changes in gas concentrations (described above) indicated that the best resolution of $\dot{V}_{O_2}$ was obtained with 7-point nearest-neighbor smoothing (i.e. the smoothed value of a given sample was the average of that sample and the three samples on either side) repeated 20 times, prior to instantaneous calculations. Considerably less noise was present in CO_2 records, but for consistency we applied the same smoothing protocol. We used 'LabAnalyst' software (Warthog Systems) to perform smoothing, baseline and lag time corrections, replace reference data by interpolation, compute $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ with Equations 2 and 3, and extract the following values for each 23.5 h recording period (approximately 24:00 h–11:30 h local time):

Daily mean $\dot{V}_{O_2}$ (daily metabolic rate; DMR), $\dot{V}_{CO_2}$, and RER (=$\dot{V}_{O_2}/\dot{V}_{CO_2}$), averaged over the 23.5 h recording period.

Minimum resting $\dot{V}_{O_2}$ averaged over 5, 10 and 30 min (RMR$_5$, RMR$_{10}$, RMR$_{30}$).

Maximum $\dot{V}_{O_2}$ averaged over 1, 2, 5 and 10 min ($\dot{V}_{O_2}1$, $\dot{V}_{O_2}2$, etc.).

Maximum wheel speed averaged over 1, 2, 5 and 10 min (V_1, V_2, etc.).

Maximum instantaneous wheel speed over a 1.5 s period (V_{max}).

Total distance run (D_{run}), total time run (T) during the 23.5 h recording period.

Mean speed (total distance/total time, V_{mean}) during the 23.5 h recording period.

Numbers (Bouts) and mean durations (D_{bout}) of running bouts (a 'bout' was defined as a period of wheel rotation lasting 3 s or more, at speeds above 0.5 r.p.m. (0.038 km h^{-1}) in either direction of rotation).

All deer mice (N=41) were measured initially at 25°C for at least 48±0.5 h (approximately noon to noon local time); some
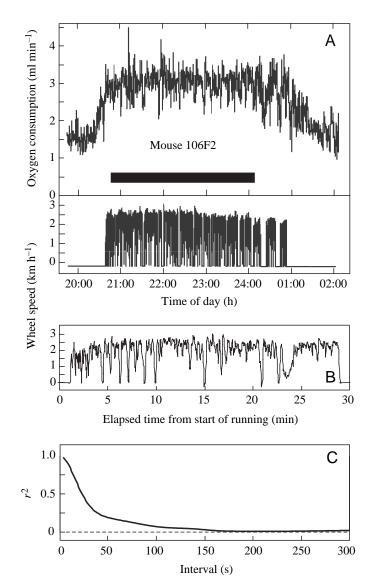

Fig. 2. (A) Oxygen consumption (top) and wheel speed (bottom) in an 18.7 g female deer mouse voluntarily exercising at 3°C. For these analyses we used absolute values of wheel speed, ignoring changes in the direction of wheel rotation. (B) First 30 min of running, showing rapid speed changes. (C) Decline in autocorrelation over increasing inter-sample intervals. The r^2 values are between samples separated by 1–200 sample intervals (1.5 s), repeated for all 8400 samples during the period indicated by the dark bar in part A (see text). Autocorrelation was negligible for intervals >150 s.

animals were measured for up to 96 h. At least 1 month after 25°C measurements, most (32) of these mice were also tested for 24 h at 10°C and again at 3°C on consecutive days. The order in which mice experienced 10°C and 3°C was random. For most comparisons, we restricted analyses to the 32 individuals used at all three T_a.

Energy cost of wheel-running

Deer mice ran at a range of speeds, so we were able to explore the relationship between speed and energy metabolism. An inherent problem in using multiple values from continuous

metabolic data is avoiding autocorrelation: metabolism does not respond instantly to changes in behavior, so repeated readings of $\dot{V}_{O_2}$ made at short intervals are not independent of each other. To define the limits to this problem, we analyzed extended sessions of running (several hundred to several thousand consecutive 1.5 s samples). Within sessions, we used the time series periodicity test in LabAnalyst to produce regressions between samples separated by 1–200 sample intervals. This procedure tests how well a value at sample k predicts the value at sample $k+i$, where i is 1–200. The inter-sample interval was 1.5 s, providing r^2 values for repeated readings over intervals of 1.5 s to 300 s (Fig. 2). Results from numerous animals showed that r^2 always decayed to very low levels (<0.02) within 150–180 s (i.e. values of $\dot{V}_{O_2}$ separated by more than 180 s were essentially uncorrelated). Accordingly, we used a 'stepped sampling' algorithm in LabAnalyst that took a series of 60 s averages of speed and $\dot{V}_{O_2}$, with the final sample in each 60 s block separated by 180 s from the start of the next 60 s block. To obtain stepped samples, we selected an extended session of running (as defined above) and identified the time of the highest 60 s mean $\dot{V}_{O_2}$ (T_{peak}) within the session. Stepped sampling began at T_{peak} and proceeded forwards and backwards in time to the beginning and end of the selected session. Thus, a session of wheel-running lasting 3.0 h would yield 45 stepped samples. To reduce potential problems associated with electrical noise and activity outside of running wheels, we discarded data with absolute wheel speeds less than 0.5 r.p.m. (0.034 km h^{-1}) averaged over the 60 s block.

Like $\dot{V}_{O_2}$, wheel rotation did not respond instantly to changes in behavior (because of the inertia of the wheel). However, the decay time for wheel rotation (defined as the time necessary for a wheel spinning at ~50 r.p.m. to slow to a stop) was 20–30 s, so the 180 s stepped sampling interval developed for $\dot{V}_{O_2}$ also eliminated autocorrelation problems in wheel speed data.

The resulting datasets were used to generate regressions of $\dot{V}_{O_2}$ vs wheel speed for each mouse (e.g. Fig. 3); when a mouse ran on more than 1 day at a particular temperature and hence provided up to 4 regressions at that temperature, we used the regression with the highest maximum speed or, if all maximum speeds were >1.3 km h^{-1} (20 r.p.m.), the highest r^2.

Maximum aerobic capacity in exercise

Maximum $\dot{V}_{O_2}$ during forced exercise ($\dot{V}_{O_2max}$) was obtained by running deer mice in an enclosed motorized treadmill, as described previously (Chappell, 1984; Chappell and Snyder, 1984; Hayes and Chappell, 1990; Chappell et al., 2003; http://biology.ucr.edu/people/faculty/MACpubs/treadmill.html). In brief, the treadmill's working section (6 cm wide, 7 cm high, 13.5 cm long) was supplied with air at 2100 ml min^{-1} STP. Mice were placed in the working section, allowed a 1–2 min adjustment period, and then run at increasing speeds, starting at 0.150.2 m s^{-1} and raised in step increments of about

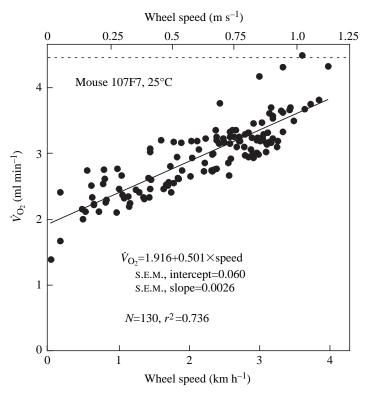Fig. 3. The relationship between voluntary running speed and oxygen consumption ($\dot{V}_{O_2}$) in a 21.5 g deer mouse tested at an ambient temperature of 25°C. This female ran 18.9 km during the 24 h measurement period. Data points are 60 s averages separated from other points by at least 3 min to avoid autocorrelation biases (Fig. 2). Data for speeds less than 0.034 km h^{-1} (0.5 r.p.m.) were not used (see text). The broken line is equal to this animal's maximal $\dot{V}_{O_2}$ during forced treadmill exercise (4.304 ml min^{-1}) and the estimated maximum aerobic speed is 4.8 km h^{-1}.

0.1 m s^{-1} every 30–45 s. A test was terminated when the mouse no longer maintained position and $\dot{V}_{O_2}$ did not increase with increasing speed; this typically occurred at 0.5–0.8 m s^{-1} (1.8–2.9 km h^{-1}). All mice showed behavioral signs of exhaustion at the end of exercise (loss of coordination, failure to maintain speed, stable or declining $\dot{V}_{O_2}$ despite speed increases) but none were injured. Reference readings of incurrent air were obtained at the start and end of measurements.

Because of the short duration of treadmill tests (most were completed with <10 min of exercise), we applied the 'instantaneous' transformation (Bartholomew et al., 1981) to resolve rapid changes in metabolism. The effective volume of the treadmill, calculated as described for wheel enclosures, was 903 ml. We calculated $\dot{V}_{O_2}$ with Equation 1 and computed $\dot{V}_{O_2max}$ as the highest instantaneous $\dot{V}_{O_2}$ averaged over continuous 1-min intervals, using LabAnalyst.

Treadmill tests were performed at room temperature (22–25°C) after 25°C wheel enclosure studies, but prior to wheel tests at 10°C and 3°C. Each individual's wheel and treadmill tests were at least 2 weeks apart.

Statistics

Since most individuals were tested for voluntary exercise under several experimental conditions (multiple days at 25°C, and 24 h at each of two other ambient temperatures), we used general linear models (GLM) for repeated measures to test for differences among variables. Individuals were experimental units, day (or temperature) was the within-subjects factor, and sex was included as a fixed factor. Analyses showed that body mass affected metabolic variables ($\dot{V}_{O_2}$), but did not influence behavioral variables (speed, distance and time spent running). Accordingly, body mass M_b was included as a covariate for metabolic variables only. Where sphericity assumptions were invalid (Mauchly's sphericity test), the Huynh–Felt degrees of freedom correction was applied in significance tests. Least-squares regression was used to describe relationships among metabolic and behavioral variables within test temperatures. Residuals from univariate ANCOVA (sex and mass as covariates) were used to assess repeatability between days or across temperatures. Several behavioral variables (D_{run}, T, V_{mean}, Bouts and D_{bout}) were log-transformed prior to analysis to provide normal distributions. The upper and lower limits to measured metabolic rates were treadmill $\dot{V}_{O_2max}$ [which scaled to $M_b^{0.789}$] and RMR at 25°C (which scaled to $M_b^{0.791}$). Accordingly, when presenting mass-adjusted results (e.g. frequency histograms for voluntary $\dot{V}_{O_2}$), we scaled data to $M_b^{0.790}$. We performed most analyses with the regression and GLM procedures in SPSS for the Macintosh (SPSS Inc.), or with Statistica/Mac (StatSoft, Inc.). The significance level (P) was 0.05. For multiple simultaneous tests, we adjusted P using a sequential Bonferroni correction (Rice, 1989).

Results

Forced exercise

In the 37 deer mice (18 females, 19 males) tested for maximal aerobic capacity during forced treadmill running, $\dot{V}_{O_2max}$ was 4.19±0.11 ml O$_2$ min^{-1} (mean ± S.E.M.) for a mouse of average mass (22.2 g). As mentioned above, mass had a strong influence on aerobic capacity even within the fairly small size range of tested mice (15.6–32.5 g; $F_{1,36}$=10.6, P=0.00254). However, sex had no effect on $\dot{V}_{O_2max}$ ($F_{1,36}$=0.055, P=0.82).

Behavior in wheel enclosures

Judging from direct observations and inferences from $\dot{V}_{O_2}$ records, deer mice in wheel enclosures were often active even when not wheel-running. For example, some animals frequently performed 'back-flips' in the mouse cage portion of the enclosure (Fig. 1). At 25°C, only 72% of the mice had been running in the wheel in the 4 min preceding the time of their highest 1 min average $\dot{V}_{O_2}$. A few mice ran less than 100 m in wheels during 24 h, while one animal (a 25.0 g male running at 10°C) covered 25.3 km in 24 h (running for a total of 14.3 h during both night and day). As we measured wheel speed and not running behavior *per se*, we do not know the fraction of measured distance that was attributable to 'coasting' (rotation caused by inertia after mice ceased running). However, for laboratory mice running in identical wheels, coasting accounted for about one third of total wheel rotations (Koteja et al., 1999a; see also Girard et al., 2001).

In light of the large behavioral variability it is unsurprising that variation in daily energy expenditure (DMR) and maximal voluntary $\dot{V}_{O_2}$ was much less than the variation in behavioral measures. Among the 32 mice tested at all three T_a, daily wheel-running distance at 25°C ranged from 0.232 to 16.5 km (mean 3.005 km) with a coefficient of variation (CV) of 124%. Running time at 25°C ranged from 17.8 to 464 min (mean 126 min, CV 82%) and maximum wheel speed ranged from 1.94 to 4.94 km h^{-1} (mean 2.93 km h^{-1}, CV 25%). In contrast, CVs were 22% for mass-adjusted DMR (mean 1.11 ml O$_2$ min^{-1} for the mean mass of 22.2 g) and 16% for mass-adjusted maximal $\dot{V}_{O_2}$ averaged over 1 min (2.97 ml O$_2$ min^{-1}). For the same animals at 3°C, CVs were 112% for distance (mean 5.55 km; range 0.027–24.5 km), 94.5% for running time (mean 197 min; range 6–707 min), 24.5% for maximum wheel speed (mean 2.57 km h^{-1}; range 1.44–4.64 km h^{-1}, 15% for DMR (mean 1.31 ml O$_2$ min^{-1}; range 1.83–3.83 ml O$_2$ min^{-1}), and 11% for 1 min $\dot{V}_{O_2}$ (mean 4.04 ml O$_2$ min^{-1}; range 3.18–4.83 ml O$_2$ min^{-1}).

Conditioning during wheel-running

In adult laboratory mice *Mus domesticus*, initial access to running wheels generally elicits successive daily increases in wheel running that last for up to 3 weeks, followed by a stabilization and eventual decline in daily wheel-running distance (e.g. Swallow et al., 2001; Morgan et al., 2003; Belter et al., 2004). We checked for such changes in deer mice using repeated-measures procedures to test for changes in 25°C running performance across days ('day' effect; Table 1). Forty-one mice experienced 2 consecutive days of wheel access and some experienced 3 or 4 consecutive days (N=16 and 7, respectively). In contrast, the 32 mice tested at 10°C and 3°C had only 1 day of wheel access at these T_a, but all had previously experienced wheels when tested at 25°C.

Although within- and between-individual variation in wheel-running at 25°C was considerable, repeated-measures ANCOVA (with body mass as covariate) found no change in DMR, minimum resting metabolic rates (RMR), or running behavior across 2, 3 or 4 days of wheel access (Table 1). However, mass affected only metabolic variables, and repeated-measures ANOVA found significant increases in mean running speed (33.1%) and maximum running speed averaged over 1, 2 and 5 min intervals (16.1%, 21.9% and 25.2%, respectively) between days 1 and 2. The number of running bouts decreased by 23.0% between days 1 and 2, but bout duration almost doubled (95.0% increase). There was a slight but statistically significant decrease (averaging about 1%) in 1–10 min average maximal $\dot{V}_{O_2}$ between days 1 and 2. Few changes occurred over days 3 and 4 in the considerably smaller subset of mice tested for more than 2 days.

Given that all mice tested at 3°C and 10°C had previous exposure to the wheels, we normally used the second day of

Table 1. *'Day' effects and repeatability of wheel-running at 25°C*

	Day effect			Repeatability		
	2 days $F_{2,37},P$	3 days $F_{2,12},P$	4 days $F_{2,3},P$	Days 1–2 $r,F_{1,30},P$	Days 1–3 $r,F_{1,13},P$	Days 1–4 $r,F_{1,4},P$
DMR	0.215,0.645	0.494,0.616	0.201,0.894	0.54,16.2,0.00013	0.13,0.231,0.319	0.39,0.718,0.222
RMR_5	2.59,0.116	0.093,0.911	0.460,0.714	0.62,23.8,0.00001	0.55,6.19,0.0131	0.71,5.06,0.0372
RMR_{30}	0.585,0.449	0.267,0.768	0.151,0.928	0.55,17.0,0.00009	0.56,6.24,0.0127	0.76,7.00,0.0228
$\dot{V}_{O_2}1$	4.75,0.0355	1.21,0.314	0.183,0.906	0.64,27.5,<0.00001	0.77,20.3,0.00025	0.74,6.20,0.0265
$\dot{V}_{O_2}2$	5.15,0.0290	1.98,0.159	0.447,0.723	0.69,35.0,<0.00001	0.76,19.1,0.00033	0.77,7.43,0.0208
$\dot{V}_{O_2}5$	6.64,0.0140	1.58,0.226	0.449,0.721	0.71,38.4,<0.00001	0.83,30.7,0.00004	0.50,1.67,0.127
$\dot{V}_{O_2}10$	4.48,0.0410	2.15,0.137	0.268,0.847	0.75,60.7,<0.00001	0.68,12.0,0.00198	0.50,1.64,0.126
D_{run}	0.0004,0.988	1.08,0.345	0.511,0.583	0.39,7.13,0.0055	0.77,20.7,0.00023	0.86,13.6,0.0070
T	2.63,0.113	0.740,0.486	0.714,0.559	0.48,11.9,0.00068	0.77,20.7,0.00023	0.84,12.3,0.0086
V_{mean}	18.1,0.00013	5.48,0.00984	1.33,0.301	0.42,8.10,0.00351	0.74,16.5,0.00058	0.30,2.11,0.103
V_{max}	2.40,0.129	1.44,0.255	0.435,0.731	0.79,63.9,<0.00001	0.86,38.2,0.00001	0.73,5.83,0.0303
$V1$	6.09,0.0181	2.44,0.105	0.623,0.611	0.67,31.9,<0.00001	0.80,24.9,0.00010	0.40,0.963,0.186
$V2$	6.57,0.0144	2.90,0.072	0.762,0.533	0.61,22.6,0.00002	0.74,17.0,0.00052	0.46,1.35,0.149
$V5$	5.61,0.0229	2.25,0.124	0.994,0.422	0.55,17.3,0.00008	0.76,18.6,0.00036	0.58,2.59,0.089
$V10$	3.83,0.058	1.56,0.227	0.987,0.425	0.57,18.6,0.00006	0.79,22.5,0.00016	0.71,5.07,0.0371
Bouts	25.8,<0.00001	1.09,0.336	5.17,0.0118	0.61,23.6,0.00001	0.48,4.10,0.031	0.916,26.2,0.00181
D_{bout}	9.36,0.0120	1.04,0.347	0.621,0.612	0.18,0.546,0.232	0.47,3.92,0.034	0.11,0.057,0.411

Metabolic variables ($\dot{V}_{O_2}$, DMR, RMR) were analyzed with repeated-measures ANCOVA (sex as a fixed factor, body mass as covariate) and behavioral variables with repeated-measures ANOVA (sex as a fixed factor; body mass had no effect). Repeatability was assessed from residuals (Pearson's r between pairs of days).

N=41 for days 1 and 2, 16 for day 3, and 7 for day 4.

DMR, daily energy expenditure; RMR_5, RMR_{30}, lowest $\dot{V}_{O_2}$ averaged over 5 or 30 min; D_{run}, distance covered in 24 h; T, time spent running in 24 h; V_{mean}, average speed; V_{max}, highest speed; $V1$, $V2$, $V5$, $V10$, highest speed averaged over 1, 2, 5 and 10 min, respectively; Bouts, number of running bouts (see text); D_{bout}, mean bout duration; $\dot{V}_{O_2}1$, $\dot{V}_{O_2}2$, $\dot{V}_{O_2}5$, $\dot{V}_{O_2}10$, highest $\dot{V}_{O_2}$ averaged over 1, 2, 5, and 10 min, respectively.

25°C data when comparing running performance across temperatures. Exceptions were made for a few mice that ran substantially less on day 2 than on day 1, or ran considerably more on day 3 or 4 than during the first 2 days; for these individuals, we used data from the day with the greatest amount of running.

Relationships among performance variables

Many metabolic and locomotor traits covaried, even after removing the effects of body mass (Table 2). The 1, 2, 5 and 10 min maximal voluntary $\dot{V}_{O_2}$ values were tightly correlated (r>0.92), as were 1, 2, 5 and 10 min maximal speeds (r>0.90), so we used only 1 min values in most analyses. At 25°C, RMR was positively correlated to maximal voluntary $\dot{V}_{O_2}$, to DMR, and to cumulative distance, run time and maximal running speeds (but not to mean running speed). Maximal voluntary $\dot{V}_{O_2}$ was correlated to cumulative distance and to maximal running speed. Unsurprisingly, there were positive correlations between cumulative distance, running time and running speed (both mean speed and short-term maximum speeds). Both the number of running bouts and mean bout duration (D_{bout}) were positively correlated to cumulative distance and running time; D_{bout} was also correlated to running speed – in other words, mice ran greater distances by increasing the duration of running bouts, the number of bouts, and by running faster.

Relationships among variables were similar at 3°C and 10°C (Table 2). For the most part, correlations among behavioral variables at both of the lower T_a closely resembled those at 25°C: distance, speed and time were strongly correlated, mice increased cumulative distance by augmenting both the number and duration of running bouts, and DMR and RMR were strongly correlated to maximal voluntary $\dot{V}_{O_2}$. However, in contrast to 25°C, correlations between metabolic variables (DMR, RMR, maximal voluntary $\dot{V}_{O_2}$) and behavioral variables (cumulative distance, running time, running speed) were not significant.

The respiratory exchange ratio (RER; $=\dot{V}_{CO_2}/\dot{V}_{O_2}$) averaged over the 23.5 h daily measurement period was not correlated to any variables except mass at 3°C (r=–0.377, P=0.0333, N=32) and DMR at 25°C (r=–0.367, P=0.0388, N=32). Daily RER averages (over about 23.5 h) were 0.895 at 3°C, 0.897 at 10°C, and 0.860 at 25°C; the 25°C RER was significantly less than RER at lower T_a ($F_{2,89}$=6.35, P=0.00265). During exercise (i.e. at the times of 1, 2, 5 and 10 min maximal voluntary $\dot{V}_{O_2}$) at the three test temperatures, the mean RER for 32 mice was always less than 0.9 (Table 3), and in only three measurements did RER slightly exceed 1.0 (all at 25°C; maximum 1.08). Repeated-measures ANCOVA (body mass as the covariate) revealed no effects of mass or sex on RER. However, temperature ($F_{2,88}$=6.27, P=0.00284) and averaging interval

Table 2. *Correlations between metabolic variables and locomotor variables*

	$\dot{V}_{O_2max}$	DMR	RMR$_5$	$\dot{V}_{O_2}1$	D_{run}	T	Bouts	D_{bout}	$V1$	V_{mean}
$\dot{V}_{O_2max}$		0.092	–0.083	0.275	–0.346	0.354	–0.239	–0.278	–0.213	–0.115
DMR	0.278		**0.869***	**0.756***	**0.465**	**0.458**	0.217	0.325	**0.438**	**0.386**
RMR$_5$	0.374	**0.637***		**0.642***	**0.394**	**0.395**	0.221	0.250	**0.393**	0.326
$\dot{V}_{O_2}1$	0.251	**0.828***	0.549		**0.370**	0.193	0.063	0.198	**0.356**	0.342
D_{run}	**–0.547**	–0.046	–0.180	–0.047		**0.957***	**0.413**	**0.820***	**0.800***	**0.746***
T	**–0.496**	0.034	-0.190	–0.051	**0.944***		**0.610***	**0.667***	**0.651***	**0.531**
Bouts	–0.147	0.267	–0.019	0.108	**0.454**	**0.643***		–0.098	0.067	–0.171
D_{bout}	**–0.571**	–0.238	–0.220	–0.172	**0.849***	**0.685***	–0.040		**0.776***	**0.883***
$V1$	**–0.461**	0.099	–0.060	0.138	**0.809***	**0.652***	0.197	**0.717***		**0.874***
V_{mean}	**–0.409**	–0.048	–0.009	0.161	**0.739***	**0.490**	–0.063	**0.828***	**0.852***	
DMR	**0.356**									
RMR$_5$	0.269	**0.632***								
$\dot{V}_{O_2}1$	0.247	**0.726***	0.598							
D_{run}	**–0.501**	–0.101	–0.232	–0.133						
T	**–0.520**	–0.042	–0.257	–0.209	**0.941***					
Bouts	–0.201	0.224	–0.021	–0.198	**0.406**	**0.600***				
D_{bout}	**–0.484**	–0.273	–0.305	–0.118	**0.823***	**0.718***	–0.119			
$V1$	**–0.379**	–0.097	–0.098	–0.153	**0.771***	**0.693***	0.196	**0.710***		
V_{mean}	**–0.405**	–0.154	–0.154	–0.028	**0.843***	**0.672***	–0.065	**0.905***	**0.825***	

Upper right: 25°C data; middle left: 10°C data, bottom; 3°C data (*N*=32 for all).

r, correlation; $\dot{V}_{O_2max}$, maximal oxygen consumption during forced treadmill exercise; other abbreviations as in Table 1.

Body mass significantly affected all metabolic variables ($\dot{V}_{O_2max}$, DMR, RMR, and $\dot{V}_{O_2}1$) and *r* values for these variables are partial correlation coefficients from multiple regressions including mass.

Significant unadjusted correlations are indicated in **boldface**; absolute values of *r*>0.355 are significant to 0.05; absolute *r*>0.45 are significant to 0.01. After a sequential Bonferroni correction for multiple simultaneous tests within sub-tables (Rice, 1989), absolute values of *r*>0.61 remain significant at the 'tablewide' *P* of 0.05 (indicated with asterisks).

($F_{3,88}$=5.74, *P*=0.00228) had small but significant effects: RER was higher at 3°C and 10°C than at 25°C, and also tended to increase as the measurement interval increased.

Temperature effects and locomotion energetics

As expected, T_a strongly affected most aspects of energy metabolism (Tables 4, 5; Fig. 4A). RMR, DMR and maximal voluntary $\dot{V}_{O_2}$ were all significantly higher at low T_a than at 25°C. However, T_a did not affect mean or maximum running speeds. The highest instantaneously attained speeds (i.e. a single 1.5 s sample) were 4.73, 4.99 and 4.64 km h^{-1} at 25°C, 10°C and 3°C, respectively (the three maxima came from different mice), and the corresponding highest 1 min averages

were 4.27, 4.00 and 4.24 km h^{-1} (again, each value came from a different individual).

The relationship between running speed and power output (measured as $\dot{V}_{O_2}$) was affected by temperature but not by body mass (Fig. 4B; Table 6). Some individuals ran poorly in wheels, so to be included in the analysis a mouse's $\dot{V}_{O_2}$ *vs* speed regression had to have (i) a significant positive correlation, (ii) at least 10 data points at speeds >0.5 r.p.m. (about 0.01 m s^{-1}) and (iii) a maximum speed >0.2 m s^{-1} (0.7 km h^{-1}). We analyzed data in two ways: with univariate ANCOVA (to include all mice that ran well, *N*=26 at 25°C, 22 at 10°C, and 17 at 3°C), and with repeated-measures ANCOVA for the 16 mice that provided data at all three T_a. Both methods yielded

Table 3. *Respiratory exchange ratios (RQ) obtained at the time of maximal voluntary* $\dot{V}_{O_2}$ *(1, 2, 5 and 10 min averages) at the three test temperatures*

	Averaging interval (min)			
Temperature (°C)	1	2	5	10
3	0.838±0.076	0.858±0.053	0.877±0.052	0.885±0.048
10	0.831±0.077	0.852±0.073	0.875±0.045	0.880±0.037
25	0.787±0.084	0.816±0.097	0.821±0.084	0.840±0.089

RQ= $\dot{V}_{CO_2}/\dot{V}_{O_2}$.

N=32 individuals (14 females, 18 males); values are means ± s.D.

Table 4. *Performance at three different ambient temperatures*

	Ambient temperature (°C)		
	25	10	3
DMR (ml O_2 min^{-1})	1.147±0.26	2.15±0.32	2.48±0.36
RMR_5 (ml O_2 min^{-1})	0.464±0.091	1.16±0.46	1.31±0.20
RMR_{30} (ml O_2 min^{-1})	0.542±0.106	1.32±0.168	1.56±0.23
D_{run} (m)	3005±3720	6289±6583	5548±6229
T (min)	125.8±103.0	264.3±215.9	197.4±186.6
V_{mean} (km h^{-1})	1.35±0.53	1.23±0.55	1.23±0.67
V_{max} (km h^{-1})	2.93±0.74	2.65±0.62	2.57±0.63
$V1$ (km h^{-1})	2.26±0.89	2.12±0.70	2.08±1.00
$V2$ (km h^{-1})	2.02± 0.88	1.90±0.73	1.88±0.98
$V5$ (km h^{-1})	1.65±0.86	1.69±0.73	1.43±0.86
$V10$ (km h^{-1})	1.30±0.84	1.49±0.72	1.11±0.77
Bouts	359.9±193.7	657.8±98.4	399.5±68.5
D_{bout} (s)	21.4±20.8	24.7±16.7	38.4±48.5
$\dot{V}_{O_2}1$ (ml O_2 min^{-1})	3.06±0.57	3.68±0.64	4.04±0.44
$\dot{V}_{O_2}2$ (ml O_2 min^{-1})	2.94±0.57	3.56±0.57	3.90±0.46
$\dot{V}_{O_2}5$ (ml O_2 min^{-1})	2.79±0.57	2.40±0.57	3.66±0.65
$\dot{V}_{O_2}10$ (ml O_2 min^{-1})	2.60±0.56	3.31±0.56	3.66±0.46

Values are means ± S.D.

This data set includes a single day's results for each of 32 individuals tested at all three ambient temperatures (see text). The minimum running bout duration was set at 3 s (2 sample intervals), and running speeds less than 0.5 r.p.m. (0.038 km h^{-1}) were not counted when computing distance, run time or bout characteristics in order to minimize noise.

Abbreviations as in Tables 1 and 2.

Table 5. *Temperature effects and cross-temperature repeatability of wheel-running performance*

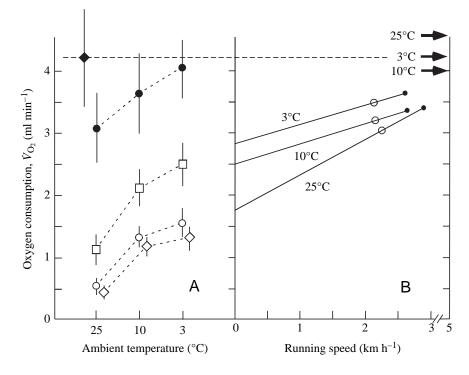|				Repeatability		
	Temperature $F_{2,58},P$	Sex $F_{1,30},P$	Interaction $F_{2,58},P$	25–10°C $r,F_{1,30},P$	25–3°C $r,F_{1,30},P$	10–3°C $r,F_{1,30},P$
DMR	10.5,0.00012	1.70,0.202	4.64,0.0135	0.40,5.66,0.012	0.56,14.0,0.00039	0.60,16.6,0.00016
RMR_5	8.35,0.00065	4.27,0.0479	3.45,0.0384	0.29,2.69,0.056	0.26,2.19,0.075	0.56,13,7,0.00043
RMR_{30}	10.3,0.00014	4.18,0.0502	4.93,0.0105	0.11,0.387,0.270	0.21,1.36,0.251	0.39,5.32,0.0141
$\dot{V}_{O_2}1$	8.51,0.00150	2.09,0.159	2.20,0.132	0.15,0.648,0.214	0.38,5.04,0.0161	0.67,24.7,0.00001
$\dot{V}_{O_2}2$	8.70,0.00120	2.34,0.137	2.30,0.121	0.14,0.589,0.229	0.32,3.35,0.0385	0.68,25.5,0.00001
$\dot{V}_{O_2}5$	8.98,0.00112	2.96,0.0958	2.89,0.0760	0.09,0.22,0.322	0.26,2.22,0.073	0.71,29.9,0.00001
$\dot{V}_{O_2}10$	6.80,0.00457	2.54,0.122	2.65,0.0918	0.14,0.632,0.216	0.37,4.83,0.0179	0.70,28.1,0.00001
D_{run}	1.06,0.352	0.179,0.675	0.709,0.496	0.21,1.42,0.122	0.23,1.66,0.104	0.68,26.3,0.00001
T	1.12,0.334	0.075,0.786	1.72,0.187	0.49,9.50,0.00219	0.37,4.78,0.0184	0.61,18.0,0.00009
V_{mean}	0.934,0.399	0.238,0.629	0.433,0.651	0.31,3.17,0.0425	0.31,3.17,0.0425	0.30,2.94,0.0485
V_{max}	0.142,0.868	0.031,0.861	0.868,0.425	0.30,2.94,0.0485	0.25,1.96,0.0875	0.63,20.2,0.00005
$V1$	0.068,0.935	0.026,0.872	0.659,0.508	0.34,3.85,0.0295	0.27,2.37,0.067	0.67,24.3,0.00001
$V2$	0.065,0.937	0.166,0.687	0.433,0.650	0.35,4.14,0.0251	0.29,2.80,0.0503	0.64,21.2,0.00004
$V5$	0.094,0.910	0.114,0.738	0.513,0.601	0.33,3.77,0.031	0.35,4.25,0.0239	0.61,18.1,0.00009
$V10$	0.344,0.710	0.142,0.709	0.359,0.700	0.32,3.49,0.0355	0.35,4.22,0.0244	0.63,19.5,0.00006
Bouts	0.146,0.865	0.163,0.689	0.311,0.734	0.45,7.47,0.052	0.28,2.58,0.059	0.35,4.19,0.0249
D_{bout}	1.56,0.223	0.933,0.342	1.29,0.279	0.42,6.43,0.0083	0.32,3.40,0.0375	0.72,32.6,<0.00001

Temperature effects were analyzed using repeated-measures ANCOVA, with sex as a fixed factor and body mass as a covariate. Repeatability was assessed from residuals of univariate ANCOVA with sex and body mass, using a one-tailed test.

All mice (N=14 females and 18 males) were tested at each of three temperatures (25°C, 10°C, 3°C).

Abbreviations as in Tables 1 and 2.

similar results and we present the repeated-measures statistics here and in Table 6. At 10°C, the regression between speed and $\dot{V}_{O_2}$ had a higher intercept and lower slope than at 25°C ($F_{1,14}$=43.4, $P<0.00001$ for intercept; $F_{1.14}$=17.0, $P=0.00078$ for slope). Regressions were more similar at 3°C and 10°C (Fig. 4B). The intercept at 3°C was higher than at 10°C

Fig. 4. (A) Minimal resting metabolism (diamonds, 5 min averages; open circles, 30 min averages), average daily energy use (squares), and highest oxygen consumption (filled circles; 1 min average) at three ambient temperatures. The solid diamond in the upper left indicates the maximal $\dot{V}_{O_2}$ (1 min average) during forced treadmill exercise at room temperature. Values are means ± S.D. (for N, see text). (B) Averaged least-squares regression lines for animals of standard mass (22.2 g) for the relationship between running speed and oxygen consumption. The rightmost end of each regression line indicates the mean maximum instantaneous running speed for that temperature and the circle indicates the mean maximum 1 min average (neither was significantly affected by temperature). Arrows indicate the estimated $\dot{V}_{O_2}$ at the highest attained instantaneous running speed of about 5 km h^{-1}.

($F_{1,14}$=35.4; P<0.00001), but slopes and 3°C and 10°C did not differ ($F_{1,14}$=0.102, P=0.754). Regression coefficients (r^2) declined at low T_a; a repeated-measures ANCOVA including slope as covariate indicated that this was partly a temperature effect ($F_{2,60}$=4.47, P=0.016) but primarily resulted from the decrease in slope at low T_a ($F_{1,60}$=11.6, P=0.00121).

For the 16 deer mice that had good running performance at all three T_a, we used each individual's slope, intercept and treadmill $\dot{V}_{O_2max}$ to estimate its maximum aerobic running speed (MAS, the speed at which $\dot{V}_{O_2max}$ is attained; Fig. 3) as:

$$MAS = (\dot{V}_{O_2max} - intercept) / slope . \quad (4)$$

Ambient temperature affected MAS, which averaged 5.45±0.50 km h^{-1} at 25°C, 6.53±0.66 km h^{-1} at 10°C, and

Table 6. *Repeated-measures ANOVA showing effects of temperature on regressions between running speed and oxygen consumption, with sex as a cofactor in the analysis*

	Slope	Intercept	r^2
Value at 25°C	0.582±0.080	1.79±0.101	0.582±0.040
Value at 10°C	0.303±0.019	2.51±0.108	0.414±0.036
Value at 3°C	0.293±0.026	2.92±0.081	0.394±0.035
Temperature (F, P)	8.17, 0.0037	55.7, <0.00001	8.57, 0.0012
Sex (F, P)	0.824, 0.379	3.93, 0.067	0.176, 0.682
Interaction (F, P)	0.268, 0.707	3.78, 0.035	0.778, 0.469

Running speed = km h^{-1}; oxygen consumption= ml O$_2$ min^{-1}.

Body mass had no significant effect on slope, intercept or r^2 at any ambient temperature.
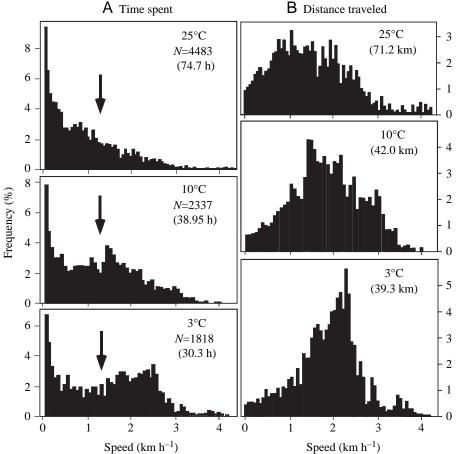
Of 32 mice tested, 16 (8 males, 8 females) ran with enough consistency to yield useable regressions at all three test temperatures.

Slope, intercept and r^2 are shown as means ± S.E.M.

4.70±0.65 km h^{-1} at 3°C (repeated-measures ANCOVA, $F_{2,26}$=4.07, P=0.029), with an overall mean of 5.56±0.47 km h^{-1}. Sex did not affect MAS, but there was a marginally significant interaction between T_a and mass ($F_{2,26}$=3.76, P=0.037). Using means of $\dot{V}_{O_2max}$, slope and intercept (Table 6), the MAS for a mouse of average mass (22.2 g) is 4.12 km h^{-1} at 25°C, 5.54 km h^{-1} at 10°C, and 4.33 km h^{-1} at 3°C (overall mean 4.65 km h^{-1}).

Deer mice shifted their preferred running speeds according to T_a (Fig. 5A). A well-defined peak at low speeds (~0.1–0.3 km h^{-1}) was seen at all T_a, probably attributable to the inertia of the wheel leading to slow starting or ending of rotation. At higher speeds (>0.5 km h^{-1}), preferred running speeds increased as T_a decreased. At 25°C, the distribution of running speeds resembled a simple declining function from the low-speed peak, with a weakly defined second peak at about 0.8 km h^{-1}. However, at 10°C mice running faster than 0.5 km h^{-1} preferred speeds between 1 and 2 km h^{-1}, and at 3°C the preferred range was between 1.8 and 2.6 km h^{-1}.

The distance traveled at different speeds also varied with T_a (Fig. 5B; Table 3). Despite the large amount of wheel rotation at <0.5 km h^{-1} (Fig. 5A), deer mice did not move very far at these speeds. At 25°C, mice used a broad range of speeds (0.5–3 km h^{-1}) to cover most of the distance they traveled. At lower T_a mice did most of their traveling within narrower and higher speed ranges, with peaks at 1.4–2 km h^{-1} at 10°C and about 2.5 km h^{-1} at 3°C. 75% of total distance run at 3°C was done at speeds of 1.6 km h^{-1} or higher; the corresponding values were 1.2 km h^{-1} at 10°C and 1.0 km h^{-1} at 25°C.

We used stepped sampling (60 s averages separated by 3 min) across the entire daily sampling period (about 23.5 h) to obtain distributions of voluntary $\dot{V}_{O_2}$. Data were adjusted to

A Time spent   B Distance traveled

Fig. 5. The distribution of voluntary running speeds in deer mice tested at different ambient temperatures (T_a), expressed as time spent (A) and distance traveled (B) at different speeds. Data are 1 min averages, with different points from each animal separated by at least 3 min (see text). The same 32 animals were tested at each T_a, although some individuals ran very little at particular T_a and the number of data points varied among individuals. Downward-pointing arrows (in A) indicate the mean running speed for each T_a, calculated as (cumulative distance/cumulative run) time in 24 h (Table 4). The bin size was 0.068 km h^{-1} (1 r.p.m. of wheel rotation). For clarity, speeds under 0.068 km h^{-1} are not shown.

the average body mass of 22.2 g using a scaling factor of mass$^{0.79}$ (see Statistics). At 25°C, the distribution of voluntary $\dot{V}_{O_2}$ was unimodal, with a large peak around 0.6 ml O$_2$ min^{-1} and a gradual decline in frequency at higher $\dot{V}_{O_2}$ (Fig. 6). However, the distribution was strongly bimodal at 10°C (with peaks at 1.3 and 2.6 ml O$_2$ min^{-1}) and at 3°C (with peaks at 1.7 and 3.0 ml O$_2$ min^{-1}). Few voluntary $\dot{V}_{O_2}$ exceeded treadmill $\dot{V}_{O_2max}$ at any T_a, but the fraction of data exceeding $\dot{V}_{O_2max}$ was higher at 3 and 10°C than at 25°C, in both number of samples (χ^2=94.3, d.f.=2, P<0.0001; 1.3% of 9666 samples at 3°C, 0.9% of 10292 samples at 10°C, and 0.1% of 9472 samples at 25°C) and in numbers of individuals with $\dot{V}_{O_2}$ above $\dot{V}_{O_2max}$ (χ^2=9.96, d.f.=2, P<0.01; 10/32 at 3°C, 4/32 at 10°C, and 1/32 at 25°C).

Behavioral and metabolic repeatability

In multi-day tests at constant warm temperatures, nearly all behavioral and metabolic variables were highly repeatable (Table 1). Repeatability declined by days 3 and 4, but this was partially a result of small sample size over those intervals (only 16 and 7 individuals, respectively). The main exception was the mean length of running bouts, which showed no repeatability over any interval.
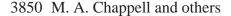
Repeatability was also high across temperatures, but only for tests at 10 and 3°C, which were made on sequential days (Table 5). As for sequential-day comparisons at 25°C, all

metabolic variables and most behavioral variables were significantly repeatable between 10 and 3°C. Repeatability between initial measurements at 25°C at measurements at 3 and 10°C (performed at least 1 month apart) was lower. Nevertheless, many traits remained significantly repeatable over the larger interval.

Maximum $\dot{V}_{O_2}$ *during forced* vs *voluntary running*

Maximal voluntarily attained $\dot{V}_{O_2}$ during wheel-running were substantially higher at low T_a than at 25°C (Tables 4, 5; Fig. 4). Nevertheless, even at 3°C, voluntarily attained maximal $\dot{V}_{O_2}$ averaged significantly less than the $\dot{V}_{O_2max}$ elicited during forced treadmill exercise. Ratios between maximal 1 min voluntary $\dot{V}_{O_2}$ and treadmill $\dot{V}_{O_2max}$ (also a 1 min average) declined significantly from 0.933±0.168 at 3°C to 0.869±0.174 at 10°C to 0.716±0.133 at 25°C ($F_{2,92}$=16.4, P<0.00001). At all T_a, 1 min voluntary $\dot{V}_{O_2}$ was significantly lower than treadmill $\dot{V}_{O_2max}$ (paired t-tests: t=2.68, P=0.0118 at 3°C; t=3.94, P=0.00043 at 10°C, and t=9.27, P<0.0001 at 25°C, N=32 for all T_a). Results were qualitatively similar for longer averaging periods (2, 5 and 10 min maximal voluntary $\dot{V}_{O_2}$).

A few deer mice did attain maximal voluntary $\dot{V}_{O_2}$ that exceeded treadmill $\dot{V}_{O_2max}$ (e.g. Fig. 3), but these high $\dot{V}_{O_2}$ values were not sustained for long periods. The highest ratios of maximal 1 min voluntary $\dot{V}_{O_2}$/treadmill $\dot{V}_{O_2max}$ in individual
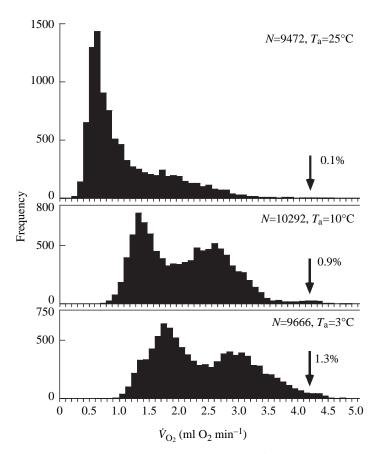
Fig. 6. The distribution of oxygen consumption ($\dot{V}_{O_2}$) during voluntary wheel-running in 32 deer mice tested at different ambient temperatures (T_a). Data are 1 min averages, with different points from each animal separated by 3 min (see text). The same animals were tested at each T_a (variation in sample sizes reflects slight differences in the number of data points in each sample period, and in data lost due to equipment problems). All $\dot{V}_{O_2}$ values were adjusted to the mean body mass of 22.2 g using a scaling factor of mass$^{0.79}$ (see text). Downward-pointing arrows indicate the treadmill-elicited maximum $\dot{V}_{O_2}$ ($\dot{V}_{O_2max}$) for a 22.2 g mouse (4.2 ml O_2 min^{-1}) and the associated percentage is the fraction of data exceeding $\dot{V}_{O_2max}$. The bin size was 0.1 ml O_2 min^{-1}.

mice were 1.07 at 25°C, 1.27 at 10°C and 1.38 at 3°C. These three maxima were reached by different animals; the 3°C animal (an 18 g female) had an unusually low treadmill $\dot{V}_{O_2max}$ (her voluntary $\dot{V}_{O_2}$/treadmill $\dot{V}_{O_2max}$ ratio would have been 1.10 had she achieved the predicted $\dot{V}_{O_2max}$ for a deer mouse of her mass, based on mass regressions for our mice).

Discussion

We used specialized metabolic chambers, low-noise gas analyzers, and computer processing to achieve fine-scale temporal resolution of brief events in a system that gave our mice considerable behavioral latitude. This allowed us to explore relationships between voluntary running behavior and energy costs in unprecedented detail. Most of our deer mice adapted quickly to the wheel enclosures, running up to 25 km

per night. Despite large variance in wheel-running parameters (e.g. Table 4), we observed few indications of conditioning to the wheel ('day' effect; Table 1). High repeatability of both behavior and metabolic variables (Table 1) indicates that despite substantial population variability, individuals behaved consistently in multiple days of testing at both high (Table 1) and low (Table 5) ambient temperatures. The repeatabilites we found are consistent with those of other metabolic variables in rodents, such as food consumption and assimilation in cold-exposed laboratory mice (Koteja et al., 2000), and $\dot{V}_{O_2max}$ in deer mice (Hayes and Chappell, 1990) and ground squirrels (Chappell et al., 1995).

Metabolism and temperature

The general metabolic response of deer mice to changing ambient temperature was as expected for a small endotherm: compared to 25°C (approximately the lower critical temperature of *P. maniculatus*; Chappell and Holsclaw, 1984; Chappell, 1985), mice spent more energy overall as T_a decreased to 10°C and 3°C. Because our mice did not have access to materials that could be formed into well-insulated nests, minimal resting metabolism (RMR) was also inversely related to T_a. At 25°C, the RMR we observed for a deer mouse of the average mass of 22.2 g (0.53 ml O_2 min^{-1}; 30 min average) was slightly less than the previously reported basal metabolism for this population (about 0.6–0.7 ml O_2 min^{-1}; Chappell et al., 2003), but RMR increased 2.5-fold at 10°C and 2.97-fold at 3°C. The change in DMR was less pronounced, with the value at 10°C and 3°C elevated by 1.87-fold and 2.16-fold, respectively, over the 25°C DMR. For a 22.2 g mouse, the difference between DMR and RMR – presumably the energy spent on activity – rose from 0.603 ml O_2 min^{-1} at 25°C to 0.815 ml O_2 min^{-1} at 10°C and 0.926 ml O_2 min^{-1} at 3°C ($F_{2,89}$=15.8, $P<0.00001$).

For our non-reproductive deer mice, DMR largely comprised resting metabolism and the energy costs of exercise (plus an unknown but probably minor contribution from energy used for processing food). Therefore, it is unsurprising that DMR was positively correlated to the amount of wheel-running activity (distance run and time spent running) at 25°C (Table 2). However, wheel-running activity was not significantly correlated to DMR at the two lower T_a. This may be attributable to the 2.5- to 3-fold higher RMR at 10 and 3°C and the lower slope of the relationship between speed and $\dot{V}_{O_2}$ at low T_a (Fig. 4). The combination of these factors reduces the proportional difference between RMR and $\dot{V}_{O_2}$ at the mean running speed of about 1.3 km h^{-1} (which did not vary with T_a; Fig. 5). Also, variance in speed and running time was greater at low T_a than at 25°C (Table 4).

Energetics of locomotion

Increased expenditure on activity at low T_a is consistent with the observation that mice spent more time running and covered

greater distances at 10°C and 3°C than at 25°C (Tables 4 and 5). Taken together, these data also hint that energy costs of thermoregulation and locomotor exercise are not completely substitutive (although this argument is tenuous, since many mice engaged in exercise outside of the running wheels). Regressions of running speed *vs* metabolism (Fig. 4, Table 6) clearly reveal partial substitution of exercise heat for thermogenic heat, as indicated by significantly lower slopes at 10°C and 3°C than at 25°C (complete additivity would result in all three regressions having the same slope, and complete substitution would be indicated by slopes of zero at low T_a, at least for low to moderate speeds). Interestingly, the so-called 'postural cost' of exercise – the difference between resting metabolism and the zero-speed intercept of the speed *vs* cost regression (Taylor et al., 1970, 1982) – did not change significantly with T_a, averaging 1.25 ml O_2 min^{-1} for a 22.2 g mouse ($F_{2,60}$=2.45, P=0.095).

How does the relationship between speed and power output during voluntary exercise compare to the corresponding relationship for forced exercise? We are not aware of any published data on treadmill-derived locomotor energetics of deer mice, but considerable information exists for other small rodents (particularly wild house mice and laboratory mice, *Mus musculus* and *M. domesticus*). A widely cited early study by Taylor et al. (1970) yielded an incremental cost of locomotion (i.e. the slope of the speed *vs* power relationship) of 1.4 kJ km^{-1} (about 70 ml O_2 km^{-1} assuming 20.1 J ml^{-1} O_2) for 21 g house mice over a fairly narrow speed range (maximum speed <1 km h^{-1}). Taylor et al. (1982) provided an allometry for the incremental cost of terrestrial locomotion in relation to body mass, based on a number of studies of various birds and mammals. Their equation (kJ km^{-1}=10.7×mass in kg$^{0.684}$) predicts a slope of 0.79 kJ km^{-1} for a 22.2 g animal. By comparison, the slope we found for 22.2 g deer mice at 25°C was 0.70 kJ km^{-1} (34.9 ml O_2 km^{-1}; Table 6), half the value reported by Taylor et al. (1970) for mice, but reasonably close to the allometrically predicted slope.

We emphasize that comparisons between wheel and treadmill data are complex and should be regarded with caution, for several reasons. First, for all such studies with small endotherms, temperature may be important in determining slopes and intercepts (as our results reveal), and thermal conditions are sometimes unspecified in papers describing locomotor costs. We presume that in such cases tests were carried out at normal room temperatures (20–22°C), which for many small rodents is below the thermal neutral zone (however, temperatures within treadmill chambers may have risen to substantially higher values). Second, in treadmill studies exercise costs are usually steady-state values obtained during sustained running at constant speeds, while our mice typically ran in short bouts (Fig. 2, Table 4) and speeds were seldom constant for periods of more than a few seconds. Third, speed data from large wheels as we used are likely to be biased [from mice 'coasting', because wheels continue to turn from momentum for several seconds after mice cease running and exit (Koteja et al., 1999a), and because inertia prevents wheels

from accelerating as fast as an unhindered mouse might]. Fourth, animals in wheels can change between uphill, level and downhill running, depending on where they position themselves. Finally, the intermittency of typical voluntary running, with numerous short running bouts interspersed with brief rest periods (e.g. Fig. 1; Girard et al., 2001), could conceivably affect the metabolic data because of 'excess' post-exercise oxygen consumption (Baker and Gleeson, 1998). Despite these caveats, our results suggest fairly close correspondence between voluntary and forced running costs, possibly with voluntary costs being slightly lower.

The only comparable study of wheel-running energetics is that of Koteja et al. (1999b). These authors used food intake coupled with measures of wheel rotation in a regression model to estimate energy expenditures of laboratory mice running in the same wheels as used to construct the present metabolic chambers. They report slopes of 0.76 kJ km^{-1} in males (scaled from 32.2 g to the deer mouse body mass of 22.2 g with a mass exponent of 0.75) and 0.39 kJ km^{-1} in females (scaled as described for males from a mass of 25.5 g). Their value for females is considerably less than what they found in males, our findings, and the allometric predictions of Taylor et al. (1970) Koteja et al. (1999b) suggest that behavioral mechanisms accounted for the striking sex differences in their experiments. In contrast, we found no influence of sex on incremental running costs for deer mice (Table 6) and few sex effects on other behavioral parameters (Table 5).

The cost of running in wheels (excluding postural costs and RMR) was a small fraction of daily energy expenditures. At 25°C, deer mice spent about 6.3% of DMR on wheel-running. Mice covered more distance at the two lower T_a than at 25°C (Table 4). However, the slope of speed *vs* energy cost regressions were lower at low T_a (presumably because of partial substitution of exercise heat for thermogenesis; Table 6) and DMR was considerably higher because of thermoregulatory expenditures. Consequently, the fraction of DMR spent on wheel-running was lower at 10°C and 3°C (3.7% and 2.7%, respectively) than at 25°C. For comparison, Koteja et al. (1999b) estimated that wheel-running at room temperature consumed 4.4% and 7.5% of the DMR of laboratory mice running 4.4 and 11.6 km (the two values are for control lines and lines selected for increased wheel-running activity, respectively).

Given the small fraction of DMR used in running, it is worthwhile to calculate the energy savings attributable to partial substitution at low T_a. Because the relationship between speed and power was linear (e.g. Fig. 3), we calculated the energy benefits of partial substitution at 10°C and 3°C as:

$$\text{Savings} = \text{energy used} \times (\text{slope at 25°C} - \text{measured slope}) / \text{slope at 25°C} . \quad (5)$$

Accordingly, mice running at 10°C used about 48% less energy on locomotion (exclusive of postural costs and RMR) than would have been necessary without substitution, which is a saving of about 1.9% of DMR at that T_a. The corresponding values for 3°C are a 50% reduction in locomotor costs and a

1.9% reduction in DMR by substitution. It is debatable whether such a small energy savings on a daily basis would have much selective significance in nature. However, the reduction in power output while mice are running is quite substantial. At 3 km h^{-1}, the $\dot{V}_{O_2}$ of a deer mouse of average mass is $3.42 \text{ ml O}_2 \text{ min}^{-1}$ at $10°C$ and $3.80 \text{ ml O}_2 \text{ min}^{-1}$ at $3°C$. Without substitution, metabolism at 3 km h^{-1} would be $4.26 \text{ ml O}_2 \text{ min}^{-1}$ at $10°C$ and $4.67 \text{ ml O}_2 \text{ min}^{-1}$ at $3°C$. Those are substantial increases in energy costs (37% and 23%, respectively). Perhaps more significantly, absence of substitution could push $\dot{V}_{O_2}$ at 3 km h^{-1} close to or even above $\dot{V}_{O_2max}$; hence, running at this fairly routinely utilized speed (Fig. 5) would require anaerobic energy production and more rapid fatigue.

Limits to locomotor performance

Recently, Chappell and Hammond (2003) found that the maximal aerobic power output of deer mice undergoing forced treadmill exercise is constant across a wide temperature range (-16 to $20°C$), even after cold acclimation. They suggested that at low T_a (when RMR is considerably above thermoneutral values), the metabolic power available for sustained running – i.e. the difference between exercise $\dot{V}_{O_2max}$ and RMR – would be reduced unless mice can extensively substitute exercise heat for thermogenesis. The present study provides additional insight into this question. We found no indication that mean maximum voluntary speeds declined at low T_a, as might be predicted if aerobic capacity constrained exercise $\dot{V}_{O_2}$ (Figs 4, 5; Table 5). However, a few deer mice ran at speeds that engendered $\dot{V}_{O_2}$ close to (or even exceeding) their treadmill-elicited $\dot{V}_{O_2max}$ (Figs 3, 6). Also, the 1 min maximal $\dot{V}_{O_2}$ at $3°C$ was within 7% of $\dot{V}_{O_2max}$ (Fig. 4A), suggesting that constraints might have been apparent had we tested for voluntary activity at subzero temperatures (as encountered in winter by many *Peromyscus* populations; M. A. Chappell, personal observations; Wickler, 1980). If regressions for speed *vs* power are extrapolated to the highest voluntarily attained instantaneous speed of about 5 km h^{-1}, the estimated $\dot{V}_{O_2}$ are close to or slightly greater than $\dot{V}_{O_2max}$ at all T_a (Fig. 4B). Deer mice can run much faster than we recorded in our study, as their maximal sprint speed in a photocell-timed racetrack is about 13 km h^{-1} (Djawdan and Garland, 1988). Apparently, they do not utilize their capacity for high sprint speeds when running on wheels, which also true for laboratory house mice (Girard et al., 2001).

Deer mice did not select running speeds strictly on the basis of metabolic economy. Although high speeds engender the highest rates of energy utilization, they provide the lowest cost of transport (the energy cost needed to move a unit of mass a unit of distance, independent of speed; Taylor et al., 1982). Free-living golden-mantled ground squirrels *Spermophilus saturatus* apparently exploit this by preferentially traveling at speeds close to their maximal aerobic speed (Kenagy and Hoyt, 1989). In contrast, deer mice seldom used the high end of their voluntary speed range at any T_a (Fig. 5), and speeds approaching the maximal aerobic speed were very rare. Preferred speed showed

a temperature-related shift (Fig. 5), but the reason is not clear. One explanation is that partial substitution reduces the incremental cost of exercise at low T_a, making high-speed running less expensive as a fraction of DMR. More speculatively, wild deer mice may have experienced selection to minimize exposure to low T_a by moving more rapidly between sheltered locations than in warm conditions. Sustained locomotion in very cold conditions may result in hypothermia in deer mice (Chappell and Hammond, 2003).

How do the upper and lower limits of aerobic performance – thermoneutral RMR (i.e. at $25°C$) and treadmill $\dot{V}_{O_2max}$ – correlate with behavioral and metabolic indices of running activity? At $25°C$ we found positive correlations between RMR (which, at this T_a, is similar to the species' measured basal metabolism; Chappell and Holsclaw, 1984) and two measures of voluntary power output (DMR and maximal voluntary $\dot{V}_{O_2}$), and with most indices of running performance (run time and distance, bout duration, and maximal speed; Table 2). Also, DMR was strongly positively correlated to maximal voluntary $\dot{V}_{O_2}$ at all T_a. One interpretation of these results is that individuals capable of high aerobic exercise also incur high maintenance costs. Our current data do not reveal whether the association between RMR and running performance is attributable to genetic correlations among these traits or to phenotypic plasticity (e.g. the exercise conditioning resulting from extensive wheel-running may elevate RMR). Interestingly, 22 generations of selective breeding for high voluntary wheel running in laboratory house mice (Swallow et al., 1998; Garland, 2003) did not result in an increased basal metabolic rate (T. Garland, Jr, unpublished results).

As shown in Fig. 6, $\dot{V}_{O_2}$ during wheel running rarely approached treadmill-elicited $\dot{V}_{O_2max}$, regardless of temperature. Therefore, voluntary wheel-running speeds in deer mice do not appear constrained by aerobic capacity. That conclusion is consistent with the observation that voluntarily attained wheel-running speeds (Fig. 5A) are less than the predicted maximal aerobic speed of ~5 km h^{-1}. In contrast, the wheel running of selectively bred house mice may be limited by $\dot{V}_{O_2max}$ (see fig. 6 in Girard et al., 2001; Garland, 2003).

At the lower temperatures the distribution of *Peromyscus* wheel $\dot{V}_{O_2}$ shifted toward higher values and became distinctly bimodal. The left peak at all T_a reflects metabolic rates close to RMR (which increases as T_a decreases; Table 4). The second peak at $3°C$ and $10°C$, and its absence at $25°C$, presumably reflects a shift in the distribution of preferred running speeds from a declining unimodal function at $25°C$ (with a sharply defined peak at low speed) to bimodal distributions at lower T_a (Fig. 5A).

In addition, and somewhat unexpectedly, we found no correlations between the $\dot{V}_{O_2max}$ in forced exercise and any measure of voluntary $\dot{V}_{O_2}$ other than DMR at $3°C$. This further suggests that maximal aerobic capacity is not a determining factor in routine locomotor activity in deer mice – at least at temperatures of $3°C$ and above. Even more surprisingly, at low T_a we found negative correlations between $\dot{V}_{O_2max}$ and some of the behavioral indices of locomotor performance (such as mean

or maximal running speed, run time, and cumulative distance). However, these negative correlations showed little consistency between low and high T_a (Table 2), and we are unsure of their importance (see also Lambert et al., 1996). The conclusion that voluntary wheel-running speeds are not constrained by aerobic capacity (Fig. 6) is also consistent with respiratory exchange ratios during wheel-running, which were nearly always below 1.0 (Table 3). In contrast, during maximal forced treadmill exercise the RER of deer mice is substantially greater than 1.0 (Chappell, 1984).

Conclusions

In summary, we found strong influences of temperature on running energetics in deer mice, with partial substitution of exercise heat for thermogenic heat at low T_a. For free-living deer mice, which routinely experience low environmental temperatures in many parts of their range, partial substitution would permit considerable energy savings while running. That may confer a fitness advantage by permitting a greater range of speeds to be supported aerobically at low T_a. Our findings were not consistent with the hypothesis that voluntary running speeds are tightly constrained by aerobic capacity (although such a constraint might be evident at sub-zero temperatures), and mice rarely ran at the high speeds that minimize costs of transport. From a mechanistic perspective, high voluntary running performance was associated with high resting metabolism, which may be viewed as a cost of high performance capacity.

To our knowledge, this is the first report for any animal of energy costs of voluntary exercise measured with high temporal resolution over complete daily activity cycles. As discussed above, this approach provides insights into several aspects of locomotor physiology that are inaccessible with traditional forced-exercise protocols. Although somewhat demanding in terms of enclosure design, analyzer resolution and stability, and acquisition and analysis software, we believe the techniques described here should be applicable to a range of studies of locomotor behavior and energetics in small endotherms.

List of symbols and abbreviations

Bouts	number of running bouts
CV	coefficient of variation
D_{bout}	mean duration of running bouts
DMR	daily metabolic rate
D_{run}	distance run
FE_{CO_2}	fractional excurrent CO_2 concentration
FE_{O_2}	fractional O_2 concentration in excurrent air
FI_{CO_2}	fractional incurrent CO_2 concentration
FI_{O_2}	fractional O_2 concentration in incurrent air
GLM	general linear model
MAS	maximum aerobic running speed
M_b	body mass
RMR	resting metabolic rate
RER	respiratory exchange ratio

STP	Standard Temperature and Pressure
T	total time run
T_a	ambient temperature
T_{peak}	time of the highest mean $\dot{V}_{O_2}$ (in 60 s)
V	wheel speed
V_{CO_2}	rate of CO_2 consumption
V_{O_2}	rate of oxygen consumption
V_{O_2max}	maximum $\dot{V}_{O_2}$ during forced exercise

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