

Age and aerobic performance in deer mice

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Summary

Age impacts the phenotype of all multicellular animals, but lifetime changes in physiological traits are poorly understood for all but a few species. Here, we describe a cross-sectional study of age effects on body composition, aerobic performance and ventilation in deer mice *Peromyscus maniculatus*. This species lives considerably longer in captivity (in excess of 5 years) than most laboratory rodents, and the adaptational biology of its aerobic physiology is well studied. Our deer mice grew throughout life, and, as is typical for mammals, their basal metabolic rate (BMR) and maximal oxygen consumption in exercise ($\dot{V}_{O_{2max}}$) and thermogenesis ($\dot{V}_{O_{2sum}}$) increased as power functions of mass. Age did not affect BMR, but we found abrupt decreases in growth rate, $\dot{V}_{O_{2max}}$ and $\dot{V}_{O_{2sum}}$ at approximately 485 days of age, and the mass-adjusted maximal aerobic performance of old mice (5 years of age) was 20% ($\dot{V}_{O_{2max}}$) to 35% ($\dot{V}_{O_{2sum}}$) less than that of young animals. Breathing frequency (f) and oxygen extraction (E_{O_2}) also declined with age but did not change

abruptly. However, there were no consistent age-related changes in tidal volume (V_T) or minute volume (\dot{V}_{min}) after accounting for the effects of mass and $\dot{V}_{O_{2sum}}$. Age influenced several aspects of body composition (lean and fat mass). However, these changes were insufficient to explain the age-related declines in aerobic performance, suggesting that mass-specific oxidative capacity of lean tissue decreased with age. The performance changes we found could engender substantial reductions in the mobility and thermal tolerances of old deer mice. However, very few wild mice are likely to survive to ages where substantial performance decreases occur, so these declines are probably not subjected to strong selection in natural populations.

Key words: age, basal metabolism, maximal oxygen consumption, aerobic capacity, thermogenesis, ventilation, mammal, deer mouse, *Peromyscus maniculatus*.

Introduction

Non-genetic phenotypic variation within and among individuals is a pervasive theme in physiology, ecology and evolutionary biology. A familiar example is comparative physiology's long and productive tradition of exploring the influence of environmental factors (e.g. temperature, water availability and oxygen partial pressure) or conditioning regimes (e.g. diet, exercise training and cold exposure) on traits affecting thermal biology, osmoregulation, locomotor capacity, aerobic performance, etc. The ecological and evolutionary relevance of environmental influences on physiological traits (as well as the magnitude of their effects) varies considerably from species to species and from trait to trait, but one phenotypic factor common to all multicellular animals is age. Much research has focused on changes in physiology, morphology and behavior associated with the transition from juvenile stages to adulthood and on senescence effects near the end of the life span. With a few exceptions (e.g. age effects on human physiology, such as exercise and athletic performance, have been extensively documented; Stones and Kozma, 1985; Goldberg et al., 1996), comparative physiologists have generally paid less attention to age-related

changes in non-senescent adult animals. Age impacts nearly all aspects of physiology, often indirectly. For example, many animals continue to grow after attaining reproductive maturity, and this mass gain over the life span will influence the wide spectrum of physiological traits that vary with body size. However, age may have a significant physiological impact even in species with relatively determinate growth (such as humans).

As well as being interesting from a functional perspective, an understanding of age effects is relevant for field studies of adaptation – especially those that hinge on performance comparisons between contrasting environments or selective regimes – because population demographic structure is often complex and it is usually difficult to determine the age of free-living animals. Without data on age effects and demography, results from inter-population comparisons may be difficult to interpret. In this paper, we use a small mammal, the North American deer mouse *Peromyscus maniculatus*, as a model system to examine age effects on several frequently measured aspects of aerobic performance: basal metabolism (BMR), maximum aerobic capacity in exercise ($\dot{V}_{O_{2max}}$) and

thermogenesis ($\dot{V}_{O_{2\text{sum}}}$), and ventilatory traits that support the initial stages of oxygen uptake. We also explore age-related changes in body size, composition and organ mass, in order to put our data on aerobic performance into an appropriate physiological context.

Aerobic performance in deer mice has been intensively studied. Much is known about aerobic capacity changes in relation to temperature acclimation or acclimatization (Hayes and Chappell, 1986, 1990; Hayes, 1989a,b), and considerable work has focused on adaptations to oxygen availability in *P. maniculatus*, which inhabit a large altitudinal range (from below sea level to above 4000 m). Across North America, deer mouse populations show an array of polymorphisms in the α -chains of hemoglobin that are geographically correlated with altitude (Snyder 1981; Snyder et al., 1988), influence blood oxygen affinity and differentially affect aerobic performance at low and high altitude (Chappell and Snyder, 1984; Chappell et al., 1988). Field studies at a high altitude site suggest that natural selection favors high aerobic capacity in thermogenesis (Hayes, 1989a,b; Hayes and O'Connor, 1999). Deer mice live for more than five years in captivity (*Peromyscus* Genetic Stock Center, <http://stkctr.biol.sc.edu>; University of South Carolina; K. A. Hammond, unpublished data). Therefore, the potential exists for a substantial age range in wild populations, making a study of age-related changes in aerobic performance and morphology an important component of our understanding of altitude adaptation in these mice.

Materials and methods

Animals

We used deer mice (*Peromyscus maniculatus sonoriensis* Le Conte) from our laboratory colony at the University of California, Riverside (elevation 340 m). The mice were second- to fifth-generation descendents from a group of approximately 35 individuals captured near the Barcroft Laboratory in the White Mountains of eastern California (local elevation 3500–3900 m). The breeding program was managed to maximize outcrossing and there was no intentional selection, except that the founding population was serologically tested to ensure that none carried Sin Nombre virus (a dangerous pathogen). Animals were maintained in standard mouse cages at room temperature (22–24°C; the lower critical temperature of these mice is 25–30°C; Chappell, 1985). They were provided with bedding (wood shavings and cotton) and water and rodent chow *ad libitum*. All aspects of animal care were constant throughout the study.

Our experimental design was cross-sectional (i.e. we used measurements from only one age for each mouse). For most ages, we used a randomly chosen subset of the available animals in our colony, but for the oldest mice (>1300 days), we tested every available individual. If an animal was measured at two different ages (as was the case for all animals older than 1700 days), we used only the final set of measurements in analyses.

Oxygen consumption measurements

We used open-flow respirometry to determine basal and maximal exercise and thermogenic aerobic performance as rates of oxygen consumption (\dot{V}_{O_2}). Changes in O_2 concentration were measured with Ametek/Applied Electrochemistry S-3A analyzers and recorded on Macintosh computers equipped with National Instruments A-D converters and custom software (Warthog Systems, www.warthog.ucr.edu). Gas flow was regulated with Tylan and Applied Materials mass flow controllers upstream from the metabolism chambers; we used flow rates that maintained O_2 concentrations above 20.4% in all measurement conditions. Approximately 100 ml min⁻¹ of sample gas was scrubbed of CO_2 and water vapor (soda lime and drierite) and routed through the oxygen sensors. We calculated \dot{V}_{O_2} (in ml min⁻¹) as:

$$\dot{V}_{O_2} = \dot{V} \times (F_{IO_2} - F_{EO_2}) / (1 - F_{EO_2}), \quad (1)$$

where \dot{V} is flow rate [ml min⁻¹; standard temperature and pressure (STP)] and F_{IO_2} and F_{EO_2} are the fractional oxygen concentrations in incurrent and excurrent gas, respectively (F_{IO_2} was 0.2095 and F_{EO_2} was always >0.204). 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 $\pm 2\%$.

Basal metabolism

Basal metabolic rate (BMR) was measured during the animals' inactive phase (07.00 h–16.00 h local time) after overnight fasting (>10 h), which is sufficient to empty the gut (K. A. Hammond, unpublished data). Metabolism chambers (lucite boxes, volume 525 ml) were maintained at 30–32°C (within the thermal neutral zone of deer mice; Chappell, 1985) in an environmental cabinet. A small quantity of wood shavings was provided as bedding, and airflow rates were 500–600 ml min⁻¹ STP. 3-min reference readings were obtained automatically every 45 min with a solenoid manifold operated by the computer. Measurement periods were 1.5–4 h (depending on how quickly animals became quiescent), after which mice were removed from chambers and fed. We computed BMR as the lowest \dot{V}_{O_2} averaged over continuous 5-min intervals during periods when \dot{V}_{O_2} was low and stable.

Aerobic capacity in exercise

Maximum \dot{V}_{O_2} during exercise ($\dot{V}_{O_{2\text{max}}}$) 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, and the total enclosed gas volume was approximately 970 ml. We used a flow rate of 2100 ml min⁻¹ STP of dry air. To begin a test, we placed a mouse in the treadmill's working section and allowed a 1–2-min adjustment

period before starting the tread at low speed (approximately 0.3 m s^{-1}). We increased tread speed in increments of approximately 0.1 m s^{-1} every 30–60 s (depending on how well the animal ran) 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 until \dot{V}_{O_2} had clearly begun to decrease. All mice showed behavioral signs of exhaustion at the end of exercise, but none was injured. Tests lasted 6–17 min (2–13 min of tread movement). Reference readings of incurrent gas were obtained at the start and end of measurements.

Because of the relatively large volume of the treadmill and the short duration of exercise tests, we applied the ‘instantaneous’ correction (Bartholomew et al., 1981) to compensate for mixing characteristics and to resolve short-term changes (BMR and thermogenic \dot{V}_{O_2} were determined using steady-state equations). Effective volume of the treadmill respirometer, calculated from washout curves, was 903 ml. We computed $\dot{V}_{\text{O}_2\text{max}}$ as the highest instantaneous \dot{V}_{O_2} averaged over continuous 1- and 2-min intervals (Chappell and Snyder, 1984; Chappell et al., 1998).

Thermogenic capacity and ventilation

Maximal thermogenic \dot{V}_{O_2} , or summit metabolism ($\dot{V}_{\text{O}_2\text{sum}}$), was measured by exposing mice to moderately low temperatures in heliox (21% O_2 :79% He by volume). Heat loss rates are several times higher in heliox than in air (Rosenmann and Morrison, 1974), and we could quickly elicit $\dot{V}_{\text{O}_2\text{sum}}$ at ambient temperatures (T_a) between 0°C and -10°C , depending on the size and thermogenic capacity of the mouse. Use of these relatively warm temperatures minimized the risk of cold injury (no animal suffered frostbite during our study).

The metabolic chamber was constructed of lucite and contained a small amount of wood shavings. Chamber volume was 460 ml and heliox flow was 1700 ml min^{-1} STP. We started runs at a T_a of 0°C to -5°C and monitored \dot{V}_{O_2} as T_a dropped at a rate of approximately 1 deg. min^{-1} . When \dot{V}_{O_2} began to decline, or if it remained constant over a 5°C drop in T_a , we terminated the test and returned the mouse to its cage. Measurements lasted 6–15 min. As for $\dot{V}_{\text{O}_2\text{max}}$, we took reference readings at the start and end of measurements and computed $\dot{V}_{\text{O}_2\text{sum}}$ as the highest \dot{V}_{O_2} averaged over continuous 1- and 2-min intervals.

We used the heliox metabolism chamber as a whole-body plethysmograph to monitor ventilation frequency (f) and tidal volume (V_T) when animals attained $\dot{V}_{\text{O}_2\text{sum}}$. High-resistance orifices in incurrent and excurrent gas lines increased the chamber’s time constant for pressure fluctuations to a value considerably longer than the duration of inspiration and expiration. This permitted simultaneous measurements of ventilation and \dot{V}_{O_2} (Malan, 1973; Bucher, 1981). Pressure changes were detected by an Omega PX 164-010 transducer, amplified and sampled at 125 Hz by a computer, providing 10–11 data points breath^{-1} at the highest observed f . A water manometer measured the difference (0.5–0.9 kPa) between

chamber and ambient pressure. For each mouse, the system was calibrated by injecting 10–12 boluses of gas (1.0 ml) into the chamber at rates yielding pressure change kinetics similar to those during inhalation. For V_T calculations, we assumed lung temperature (T_L) was 35°C (based on body temperature measurements from a subset of individuals), ambient water vapor pressure was equivalent to saturation values (0.3–0.7 kPa at a T_a between -10°C and 0°C) and alveolar gas was 100% saturated with water vapor. Based on the accuracy of T_L estimates and calibration injections, and noise levels in the plethysmograph signal, maximal errors in computed V_T were $<8\%$. The analysis software calculated f over 20–70 sequential ventilation cycles, with a maximal error of $<2\%$ (usually $<1\%$). Minute volume (\dot{V}_{min} ; measured in ml min^{-1}) is the product of f and V_T . Oxygen extraction (E_{O_2} ; measured as a percentage) was computed as $100 \times \dot{V}_{\text{O}_2} / (F_{\text{E}_{\text{O}_2}} \times \dot{V}_{\text{min}})$.

Body composition

We obtained body composition data from a subset of animals that included 98% of the age range in the main sample (Fig. 1). After metabolic tests were complete, we anesthetized mice (0.07 ml of 65 mg ml^{-1} sodium pentobarbital injected i.p.) and used retro-orbital puncture to obtain blood samples (approximately 150 μl) in two heparinized microhematocrit tubes. Samples were centrifuged for 10 min at $1000g$, and hematocrit was calculated as the mean proportion of packed cells relative to total sample volume.

After euthanization (additional 0.1 ml of 65 mg ml^{-1} sodium pentobarbital injected i.p.), we removed all gut contents and measured wet and dry carcass mass. We also measured wet and dry masses of the heart and lungs. Fat was extracted from the dried carcass and organs using petroleum ether (Kerr et al., 1982) in a Goldfische apparatus to determine dry lean mass. We estimated absolute fat content by subtraction (dry carcass mass before extraction minus dry carcass mass after extraction) and computed relative fat content as percentage of wet carcass mass. Lean mass was estimated as the difference between wet carcass mass and absolute fat mass.

Analysis and statistics

We did not obtain complete data for all individuals (e.g. several mice remained active throughout measurements and did not provide valid BMRs). Data from animals discovered to be diseased (e.g. tumors found at dissection) were discarded. We used covariance analysis (ANCOVA; with mass and age as covariates) to test for differences among categorical variables (sex), and multiple regression to test relationships among continuous variables. Bounded data (E_{O_2} , hematocrit, fractional lean tissue and fat content) that were not normally distributed were arcsine square-root transformed prior to analysis.

The relationship between age and several morphological, metabolic and ventilatory variables appeared to change abruptly between 1 and 2 years of age (Figs 2, 3). For such data (including mass, $\dot{V}_{\text{O}_2\text{max}}$, $\dot{V}_{\text{O}_2\text{sum}}$, V_T and \dot{V}_{min}), we searched for ‘breakpoint’ ages with a piecewise regression algorithm that iteratively adjusts the breakpoint in a two-phase regression until

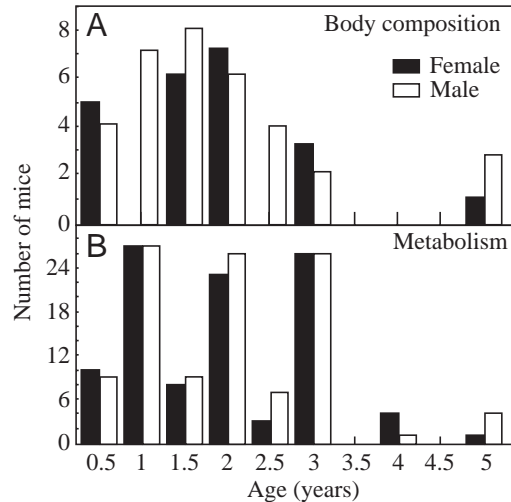


Fig. 1. Frequency distribution of sample sizes used at different ages for body composition studies (A; $N=58$) and aerobic physiology and ventilation measurements (B; $N=211$).

the overall sum of squares is maximized (Nickerson et al., 1989; Statistica instruction manual, Statsoft, Inc., Tulsa, OK, USA). The critical significance level α was 0.05; we used a sequential Bonferroni correction to adjust α in multiple simultaneous tests (Rice, 1989). Statistics were performed using JMP and Statistica software for the Macintosh (SAS Institute, Inc., Cary, NC, USA and Statsoft, Inc., respectively) and a custom-written Bonferroni correction program.

Results

We obtained metabolic and ventilation data from 211 deer mice (109 males, 102 females) ranging in age from 27 days to

1863 days (0.07–5.1 years; Fig. 1). Body composition was measured in 58 of these mice (35 males, 23 females; 27–1827 days). Due to attrition, sample sizes in the oldest age ranges were small. Nevertheless, we did not detect any subjective signs of ageing in morphology or routine behavior (e.g. changes in pelage color, gait, activity levels, etc.) once mice passed about 50 days, and even the oldest individuals appeared healthy.

Size effects

Mass was strongly correlated to BMR, $\dot{V}_{O_{2max}}$, $\dot{V}_{O_{2sum}}$, V_T and \dot{V}_{min} , but not to f or E_{O_2} (Table 1). As is typical for mammals, the metabolic rates of our deer mice scaled as power functions of body mass, with mass exponents across all ages combined of 0.762 for BMR (mean after accounting for gender differences; see below), 0.732 for $\dot{V}_{O_{2max}}$ and 0.573 for $\dot{V}_{O_{2sum}}$. However, across the body mass range in this study (10.4–41.9 g), power functions fit the data only slightly better than least-squares regressions.

Two-phase regressions between body mass and metabolism revealed inflection points at an age of approximately 485 days (Fig. 2; see below). In young mice (<485 days), mass exponents were 0.834 for BMR, 0.915 for $\dot{V}_{O_{2max}}$ and 0.812 for $\dot{V}_{O_{2sum}}$. In older mice, mass exponents decreased: 0.690 for BMR, 0.693 for $\dot{V}_{O_{2max}}$ and 0.522 for $\dot{V}_{O_{2sum}}$.

Sex differences

Our protocol was not designed to test for gender differences in life span, but there were few apparent differences in survivorship between males and females. Our small sample of the oldest animals suggests better survivorship in males: of 10 mice that reached 1350 days (3.7 years), five were females, but of the five that exceeded 1750 days (4.8 years), only one was a female.

Mice of both sexes increased body mass as they aged, and

Table 1. Effects of sex, age and body mass on mass, metabolism and ventilation during cold exposure in 211 deer mice ranging in age from 27 days to 1863 days

	Sex	Mass	Age
Mass	$F_{1,205}=24.5, P<0.000001^*$		M: $r=0.432, P=0.000004^*$ F: $r=0.521, P<0.000001^*$
BMR	$F_{1,201}=8.2, P=0.0046^*$	M: $r=0.56, P<0.000001^*$ F: $r=0.57, P<0.000001^*$	M: $r=-0.12, P=0.23$ F: $r=0.010, P=0.92$
$\dot{V}_{O_{2max}}$	$F_{1,203}=1.2, P=0.275$	$r=0.664, P<0.000001^*$	$r=-0.201, P=0.00316^*$
$\dot{V}_{O_{2sum}}$	$F_{1,202}=0.002, P=0.96$	$r=0.506, P<0.000001^*$	$r=-0.217, P=0.00209^*$
f	$F_{1,200}=7.8, P=0.00572^*$	M: $r=0.083, P=0.40$ F: $r=0.006, P=0.95$	M: $r=-0.27, P=0.00495^*$ F: $r=-0.384, P=0.000094^*$
V_T	$F_{1,198}=1.7, P=0.20$	$r=0.329, P=0.0000017^*$	$r=0.092, P=0.19$
\dot{V}_{min}	$F_{1,198}=0.002, P=0.97$	$r=0.336, P<0.000001^*$	$r=-0.071, P=0.31$
E_{O_2}	$F_{1,198}=0.13, P=0.72$	$r=0.10, P=0.14$	$r=-0.19, P=0.0070^*$

BMR, basal metabolic rate; $\dot{V}_{O_{2max}}$, maximal oxygen consumption in exercise; $\dot{V}_{O_{2sum}}$, maximal oxygen consumption in thermogenesis; f , breathing frequency; V_T , tidal volume; \dot{V}_{min} , minute volume; E_{O_2} , oxygen extraction.

Age, mass and metabolism were \log_e -transformed prior to analysis. ANCOVA with mass and age as covariates was used to test for sex differences, and multiple regression was used to examine the effects of age and mass. Separate partial correlation coefficients (r) and P values are given for males (M) and females (F) for variables where the sexes differed significantly. P values with an asterisk (*) remained significant after sequential Bonferroni correction (adjusted $\alpha=0.008$; Rice, 1989).

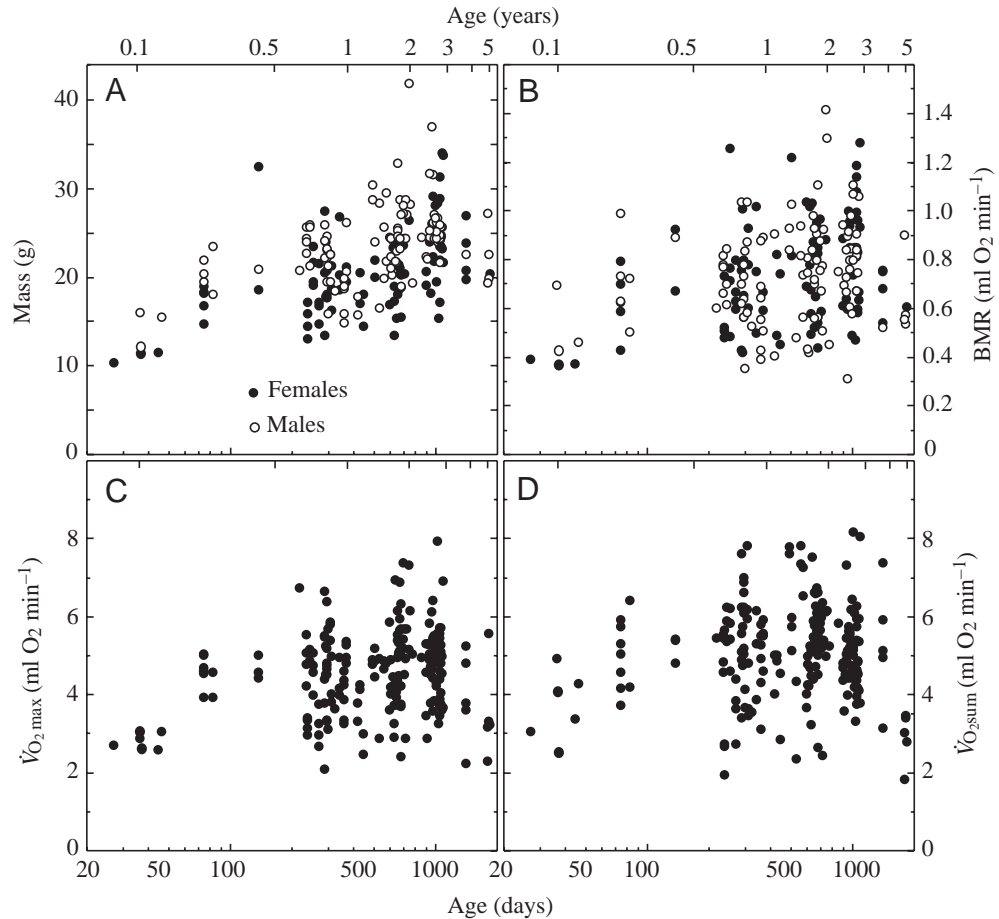


Fig. 2. Age-related changes in mass and aerobic metabolism in 211 deer mice. Different symbols are used for males and females for variables with significant gender differences. BMR, basal metabolic rate; $\dot{V}_{O_2\max}$, maximal oxygen consumption in exercise; $\dot{V}_{O_2\text{sum}}$, maximal oxygen consumption in thermogenesis.

males averaged approximately 13% heavier than females (Fig. 2; $F_{1,205}=24.5$, $P<0.00001$; ANCOVA with age as the covariate). However, after correction for age and body mass, neither hematocrit nor any measured body composition variable (heart mass, lung mass, lean mass, fat mass and fractional lean and fat content) differed significantly between males and females ($P>0.1$ in all cases).

An ANCOVA with age and body mass as covariates revealed few sex differences in metabolism and ventilation. Only BMR and f were significantly affected by gender. After correcting for age and mass, female BMRs were approximately 9% higher than those of males [0.780 ml min^{-1} and 0.716 ml min^{-1} at a standard (mean) mass of 22.4 g, respectively; $F_{1,201}=8.2$, $P=0.0046$]. Mass exponents for male and female BMR did not differ significantly. Male f was approximately 6% higher than that of females ($8.21\text{ breaths s}^{-1}$ and $7.77\text{ breaths s}^{-1}$ at standard mass, respectively; $F_{1,200}=7.8$, $P=0.0057$).

Age effects

The piecewise regression algorithm identified growth rate breakpoints at 469 days (females) and 492 days (males). In mice older than the breakpoint age, the relationship between age and mass was significant in females ($r=0.33$, $P=0.01$, with a growth rate of approximately 0.0022 g day^{-1}) but not in males ($r=0.06$, $P=0.60$). In younger mice, the corresponding

relationships were significant (females: $r=0.45$, $P=0.0024$; males: $r=0.33$, $P=0.036$), with growth rates for both sexes averaging approximately 0.0118 g day^{-1} .

Because mass changed with age, we included mass as a covariate in all analyses of age effects. There were significant age effects on both $\dot{V}_{O_2\max}$ and $\dot{V}_{O_2\text{sum}}$ but not on BMR (Table 2). Maximal \dot{V}_{O_2} decreased with age, with 5-year-old mice having approximately 22% and 30% less mass-adjusted aerobic capacity than 100-day-old mice in exercise and thermogenesis, respectively. As for growth rate, the decline in mass-adjusted maximal \dot{V}_{O_2} with age began abruptly (Fig. 2). Piecewise regression found breakpoints at 485 days and 483 days in $\dot{V}_{O_2\max}$ and $\dot{V}_{O_2\text{sum}}$, respectively (Figs 2, 4; we assume the difference in breakpoints for growth rate, $\dot{V}_{O_2\max}$ and $\dot{V}_{O_2\text{sum}}$ are statistical artefacts and not real differences). Since no animals in the data set were between 444 days and 492 days of age and all breakpoints (for mass, $\dot{V}_{O_2\max}$ and $\dot{V}_{O_2\text{sum}}$) were at or between these ages, we refer to mice aged <485 days as 'young' and mice aged >485 days as 'old'. After correction for mass changes, age was not a significant predictor of $\dot{V}_{O_2\max}$ or $\dot{V}_{O_2\text{sum}}$ in young deer mice but had a strong effect in old mice (Table 2).

Breathing frequency during maximal thermogenesis (f) was correlated with age but not with $\dot{V}_{O_2\text{sum}}$ in females, while the reverse was true in males (Table 3). Oxygen extraction (E_{O_2})

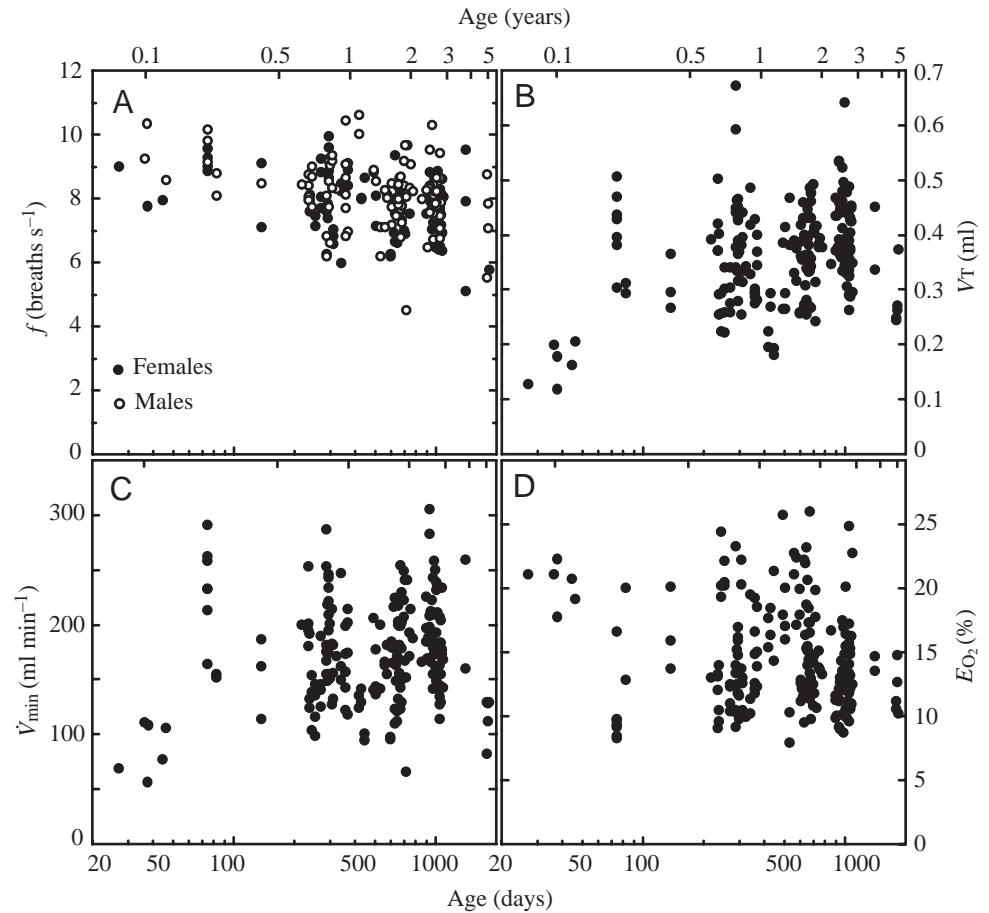


Fig. 3. Age-related changes in ventilation and oxygen extraction in 211 deer mice. Different symbols are used for males and females for variables showing significant gender differences. \dot{V}_{\min} , minute volume; V_T , tidal volume; f , breathing frequency; E_{O_2} , oxygen extraction.

Table 2. Breakpoint regression results (shown as partial correlation coefficients) for the effects of age on $\dot{V}_{O_{2\max}}$ and $\dot{V}_{O_{2\text{sum}}}$ in deer mice ranging in age from 27 days to 1863 days

	Age breakpoint	Age < breakpoint	Age > breakpoint
$\dot{V}_{O_{2\max}}$	485 days	$N=83$ Mass: $r=0.774$, $P<0.000001^*$ Age: $r=-0.103$, $P=0.36$	$N=125$ Mass: $r=0.569$, $P<0.000001^*$ Age: $r=-0.291$, $P=0.00102^*$
$\dot{V}_{O_{2\text{sum}}}$	483 days	$N=82$ Mass: $r=0.621$, $P<0.000001^*$ Age: $r=-0.046$, $P=0.68$	$N=124$ Mass: $r=0.419$, $P=0.000015^*$ Age: $r=-0.410$, $P=0.000024$

Regressions were derived from \log_e -transformed mass, age, maximal oxygen consumption in exercise ($\dot{V}_{O_{2\max}}$) and maximal oxygen consumption in thermogenesis ($\dot{V}_{O_{2\text{sum}}}$); nearly identical results were obtained when regressions were based on mass-corrected \dot{V}_{O_2} (Fig. 4). The overall r values (including the effects of both mass and age and combined across both age groups) were 0.85 for $\dot{V}_{O_{2\max}}$ and 0.805 for $\dot{V}_{O_{2\text{sum}}}$. P values with an asterisk (*) remained significant after sequential Bonferroni correction (adjusted $\alpha=0.017$).

was independent of mass but declined with age in both sexes. However, there was no apparent inflection point in the relationship between age and either f or E_{O_2} (Fig. 3). Two ventilatory variables (V_T and \dot{V}_{\min}) changed abruptly at approximately 485 days (Fig. 3). However, this may have been a consequence of the relationship between age, mass and $\dot{V}_{O_{2\text{sum}}}$; after accounting for the effects of $\dot{V}_{O_{2\text{sum}}}$ and mass, V_T and \dot{V}_{\min} were not correlated with age in either sex.

Relationships among metabolic and ventilatory indices

After correction for mass and age, BMR approached

significance as a predictor of $\dot{V}_{O_{2\max}}$ for old mice (partial $r=0.174$, $P=0.058$, $N=122$) but was not correlated with $\dot{V}_{O_{2\max}}$ in young mice or with $\dot{V}_{O_{2\text{sum}}}$ in either age class (partial $r=0.10$, $P\geq 0.15$, $N=82-204$). The mean $\dot{V}_{O_{2\text{sum}}}$ was 12% and 8% higher than $\dot{V}_{O_{2\max}}$ in young and old mice, respectively (Fig. 4), and the ratio between the two indices showed considerable variance (Fig. 5) that was not affected by either age or sex ($P=0.10$ and $P=0.15$, respectively; interaction, $P=0.50$). Nevertheless, $\dot{V}_{O_{2\max}}$ was strongly correlated to $\dot{V}_{O_{2\text{sum}}}$ in both young mice (partial $r=0.30$, $P=0.0066$, $N=82$) and old animals (partial $r=0.53$, $P<0.00001$, $N=121$).

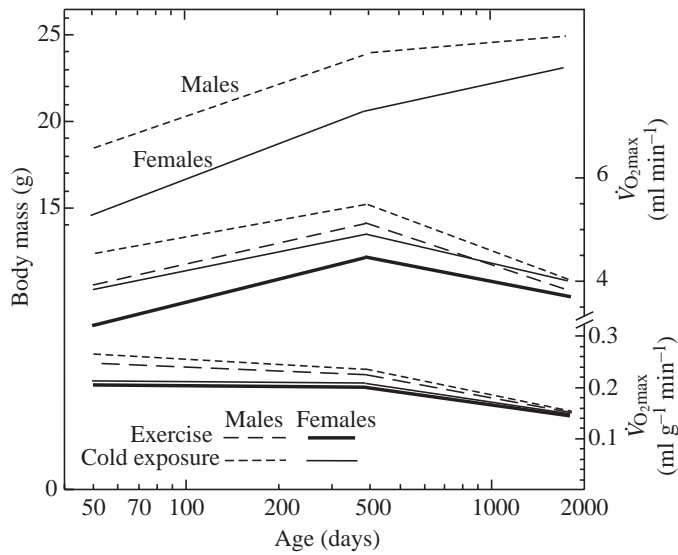


Fig. 4. Simplified representation of mean age-related changes in body mass (top), whole-animal maximal oxygen consumption (middle) and mass-specific maximal oxygen consumption (bottom) in male (broken lines) and female (solid lines) deer mice. For maximal oxygen consumption, heavy lines indicate $\dot{V}_{O_2\max}$ (exercise) and thin lines indicate $\dot{V}_{O_2\text{sum}}$ (cold exposure). Lines are derived from 'breakpoint' (piecewise) regressions based on data in Fig. 2.

Body mass did not influence V_T and \dot{V}_{\min} , but these variables were significantly correlated to $\dot{V}_{O_2\text{sum}}$ (Table 3). After correcting for age and mass, f did not affect V_T (partial $r=-0.07$, $P=0.31$, $N=199$); thus, more rapid breathing did not necessitate reduced V_T (within the measured range of f and V_T). Similarly, V_T was not correlated to lung mass after correcting for $\dot{V}_{O_2\text{sum}}$, age and body mass (partial $r=0.11$, $P=0.42$, $N=55$).

Body composition

Animals used for body composition studies showed the same general relationship between aerobic performance and age seen in the complete data set: after mass correction, age had no effect on BMR or on maximal \dot{V}_{O_2} in the 28 animals younger than 485 days. In old mice, both $\dot{V}_{O_2\max}$ and $\dot{V}_{O_2\text{sum}}$ declined significantly with age ($r=-0.382$, $P=0.041$ and $r=-0.465$, $P=0.011$, respectively).

Simple correlations with body mass were significant for heart, lung, fat mass and lean mass. Heart and lung masses were correlated, and fat content (as a percentage of body mass) was positively correlated to $\dot{V}_{O_2\max}$ in young animals and to $\dot{V}_{O_2\text{sum}}$ in old animals. However, after correction for both age and lean tissue mass, fat content and lung and heart mass were not significantly correlated to any metabolic variable except for $\dot{V}_{O_2\max}$ in young animals (Table 4).

Aside from its effects on body mass, age had no influence on body composition in young deer mice, but several aspects of body composition tended to change with age in old animals

Table 3. Combined effects of mass, age and $\dot{V}_{O_2\text{sum}}$ on breathing frequency (f), tidal volume (V_T), minute volume (\dot{V}_{\min}), and oxygen extraction efficiency (E_{O_2}) in deer mice ranging in age from 27 days to 1863 days

	Mass	Age	$\dot{V}_{O_2\text{sum}}$
f (M)	$r=-0.11$, $P=0.26$	$r=-0.201$, $P=0.042$	$r=0.435$, $P=0.000044^*$
f (F)	$r=-0.088$, $P=0.39$	$r=-0.337$, $P=0.00084^*$	$r=0.160$, $P=0.120$
V_T	$r=0.175$, $P=0.0394$	$r=0.149$, $P=0.0353$	$r=0.256$, $P=0.000259^*$
\dot{V}_{\min}	$r=0.146$, $P=0.0131$	$r=0.009$, $P=0.90$	$r=0.363$, $P<0.000001^*$
E_{O_2}	$r=-0.130$, $P=0.065$	$r=-0.101$, $P=0.125$	$r=0.413$, $P<0.000001^*$

$N=109$ males (M), 102 females (F). P values that remained significant after sequential Bonferroni correction (adjusted $\alpha=0.0045$) are indicated with an asterisk.

Table 4. Effects of body composition on metabolism in deer mice ranging in age from 27 days to 1827 days

Body composition	BMR ($N=54$)	$\dot{V}_{O_2\max}$		$\dot{V}_{O_2\text{sum}}$	
		Age < breakpoint ($N=25$)	Age > breakpoint ($N=30$)	Age < breakpoint ($N=25$)	Age > breakpoint ($N=30$)
Heart mass	$r=0.337$, $P=0.0145^*$	$r=0.642$, $P=0.00126^*$	$r=0.236$, $P=0.233$	$r=0.443$, $P=0.0387$	$r=0.333$, $P=0.0827$
Lung mass	$r=0.266$, $P=0.0565$	$r=0.604$, $P=0.00287^*$	$r=0.358$, $P=0.0612$	$r=0.420$, $P=0.0515$	$r=0.392$, $P=0.0394$
% fat content	$r=0.298$, $P=0.0320$	$r=0.601$, $P=0.00307^*$	$r=0.128$, $P=0.516$	$r=0.439$, $P=0.0408$	$r=0.424$, $P=0.0245$
Hematocrit	$r=0.280$, $P=0.0443$	$r=0.413$, $P=0.056$	$r=0.231$, $P=0.237$	$r=0.462$, $P=0.0305$	$r=0.347$, $P=0.0705$

BMR, basal metabolic rate; $\dot{V}_{O_2\max}$, maximal oxygen consumption in exercise; $\dot{V}_{O_2\text{sum}}$, maximal oxygen consumption in thermogenesis.

Age breakpoints for $\dot{V}_{O_2\max}$ and $\dot{V}_{O_2\text{sum}}$ (485 days and 483 days, respectively) were obtained from the complete data set (Table 2). Regressions were based on \log_e -transformed metabolic data and included the effects of \log_e -transformed lean tissue mass and age. Partial r and P values are shown; P values that remained significant after Bonferroni correction are indicated with asterisks (adjusted $\alpha=0.0167$ for BMR and 0.0036 for $\dot{V}_{O_2\max}$ and $\dot{V}_{O_2\text{sum}}$).

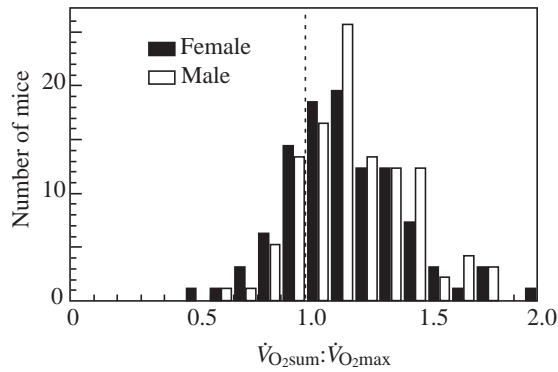


Fig. 5. The relationship between maximal oxygen consumption in thermogenesis ($\dot{V}_{O_{2sum}}$) and maximal oxygen consumption in exercise ($\dot{V}_{O_{2max}}$) in 211 deer mice. Data are normalized to $\dot{V}_{O_{2max}}$ (i.e. a value of 1.0 indicates that $\dot{V}_{O_{2max}} = \dot{V}_{O_{2sum}}$). The ratio of these two aerobic indices did not vary significantly with either sex or age.

Table 5. Effects of age and body mass on several aspects of body composition in 'old' deer mice (age >485 days)

	Body mass	Age
Lean mass	$r=0.601, P=0.00045^*$	$r=0.0941, P=0.621$
Lean mass (as % body mass)	$r=-0.592, P=0.00056^*$	$r=0.415, P=0.0227$
Fat mass	$r=0.615, P=0.00030^*$	$r=-0.406, P=0.026$
Fat content (as % body mass)	$r=0.574, P=0.00091^*$	$r=-0.457, P=0.011$
Heart mass	$r=0.614, P=0.00031^*$	$r=0.173, P=0.360$
Lung mass	$r=0.490, P=0.0060^*$	$r=0.080, P=0.675$

The table shows partial correlation coefficients (r) and P values. Asterisks indicate P values remaining significant after sequential Bonferroni correction (adjusted $\alpha=0.0071$). In younger animals, no body composition variable was significantly correlated with age after correction for body mass.

(>485 days; Table 5). Therefore, we tested whether age-related declines in aerobic performance in old mice could be explained by age-related changes in body composition. Other things being equal, maximal performance should be positively correlated to fractional lean tissue content and negatively correlated to fractional fat content. Because $\dot{V}_{O_{2max}}$ and $\dot{V}_{O_{2sum}}$ declined with age in deer mice, that hypothesis predicts a decline in fractional lean tissue content with age. However, we found a different pattern: fractional lean tissue tended to increase with age, while fractional fat content declined with age (although neither relationship was significant after Bonferroni correction; Table 5). Also, after correcting for mass and fractional fat and lean content, we found no influence of age on $\dot{V}_{O_{2max}}$, $\dot{V}_{O_{2sum}}$ or BMR for young mice or for all ages combined (Tables 6, 7), but an identical test in old mice revealed negative correlations between age and both $\dot{V}_{O_{2max}}$ and $\dot{V}_{O_{2sum}}$ (again, neither relationship was significant after Bonferroni correction). Taken together, these findings suggest

Table 6. Effects of age and body composition (lean tissue and fat) on BMR, $\dot{V}_{O_{2max}}$ and $\dot{V}_{O_{2sum}}$ in deer mice from 27 to 1827 days old

		Fat and lean (absolute values)	Fat and lean (fractions of body mass)
BMR	Fat	$r=0.312, P=0.0228$	$r=-0.096, P=0.499$
	Lean tissue	$r=0.321, P=0.0191$	$r=-0.106, P=0.454$
	Age	$r=0.150, P=0.283$	$r=0.038, P=0.787$
$\dot{V}_{O_{2max}}$	Fat	$r=0.401, P=0.00295^*$	$r=0.237, P=0.091$
	Lean tissue	$r=0.505, P=0.000113^*$	$r=0.203, P=0.149$
	Age	$r=-0.101, P=0.470$	$r=-0.227, P=0.106$
$\dot{V}_{O_{2sum}}$	Fat	$r=0.437, P=0.00107^*$	$r=-0.014, P=0.920$
	Lean tissue	$r=0.423, P=0.00161^*$	$r=-0.065, P=0.646$
	Age	$r=-0.14, P=0.315$	$r=-0.214, P=0.127$

BMR, basal metabolic rate; $\dot{V}_{O_{2max}}$, maximal oxygen consumption in exercise; $\dot{V}_{O_{2sum}}$, maximal oxygen consumption in thermogenesis.

Regressions were performed across all ages. Regression coefficients (r) and P values for fat and lean tissue content were computed from absolute tissue mass and as fractions of whole body mass; in the latter case, regressions also included whole body mass (r and P values not shown). P values significant after Bonferroni correction are indicated with an asterisk (adjusted $\alpha=0.0038$ for all ages combined).

that the decline in performance with age is not simply a result of body composition changes.

Discussion

Life span, growth and performance

Deer mice have long life spans compared with outbred strains of laboratory mice (*Mus musculus*, 1.5–3 years; Harkness and Wagner, 1989) and rats (*Rattus norvegicus*, 2–3.5 years; Baker et al., 1979). However, they live as long as expected for a generalized mammal of similar mass: an allometric regression spanning a wide range of taxa and body sizes (Calder, 1984) predicts a life span of 5.3 years in a 20 g mammal. Our oldest deer mice (which appeared healthy) were 5.1 years old. Other reports (e.g. *Peromyscus* Genetic Stock Center, <http://stkctr.biol.sc.edu>; University of South Carolina) indicate that most *Peromyscus* species (including *P. maniculatus*) have maximum life spans of 3–5 years in captivity, although white-footed mice (*P. leucopus*) may attain 8 years.

We found no change in aerobic physiology with age in young deer mice (<485 days old) other than mass scaling due to increases in body size with age. Within this age group, the three measured aerobic indices (BMR, $\dot{V}_{O_{2max}}$ and $\dot{V}_{O_{2sum}}$) had mass exponents (0.812–0.915) close to those reported in interspecific studies across a wide range of body mass (e.g. Seeherman et al., 1981; Calder, 1984). Young deer mice grew continuously, gaining about 4.8 g between 50 days and 485 days of age (a total mass increase of approximately 28%; mean for both sexes combined).

Table 7. Effects of age and body composition (lean tissue and fat) on $\dot{V}_{O_{2max}}$ and $\dot{V}_{O_{2sum}}$ in deer mice younger and older than the 'breakpoint' age of 485 days

Variable	Young mice (<485 days)		Old mice (>485 days)		
	Fat and lean (absolute values)	Fat and lean (fractions of body mass)	Fat and lean (absolute values)	Fat and lean (fractions of body mass)	
$\dot{V}_{O_{2max}}$	Fat	$r=0.509, P=0.013$	$r=0.237, P=0.289$	$r=0.481, P=0.0096$	$r=0.152, P=0.449$
	Lean	$r=0.397, P=0.060$	$r=0.174, P=0.439$	$r=0.346, P=0.0708$	$r=0.231, P=0.245$
	Age	$r=0.207, P=0.344$	$r=0.055, P=0.808$	$r=-0.264, P=0.174$	$r=-0.425, P=0.027$
$\dot{V}_{O_{2sum}}$	Fat	$r=0.360, P=0.091$	$r=0.155, P=0.491$	$r=0.121, P=0.582$	$r=-0.225, P=0.259$
	Lean	$r=0.466, P=0.025$	$r=0.132, P=0.557$	$r=0.065, P=0.767$	$r=-0.289, P=0.143$
	Age	$r=0.113, P=0.608$	$r=0.003, P=0.989$	$r=-0.370, P=0.083$	$r=-0.399, P=0.039$

$\dot{V}_{O_{2max}}$, maximal oxygen consumption in exercise; $\dot{V}_{O_{2sum}}$, maximal oxygen consumption in thermogenesis.

Regressions were performed on animals younger and older than the 'breakpoint' age of 485 days determined from the complete data set. Regression coefficients (r) and P values for fat and lean tissue content were computed from absolute tissue mass and as fractions of whole body mass; in the latter case, regressions also included whole body mass (r and P values not shown). No P value is significant after Bonferroni correction (adjusted $\alpha=0.0023$ for young and old mice).

Growth slowed in older animals (with no significant mass change in old males) but we did not see the declines in body mass that often occur in the later part of the life span in laboratory rodents (Sprott and Austad, 1996). More importantly, we found a substantial decline in mass-adjusted whole-animal performance in old mice (Fig. 4). Relative to a 100-day-old deer mouse weighing 22.5 g, a 5-year-old individual of the same mass has approximately 22% less aerobic capacity in exercise ($\dot{V}_{O_{2max}}=4.92 \text{ ml O}_2 \text{ min}^{-1}$ vs $3.84 \text{ ml O}_2 \text{ min}^{-1}$) and 30% less aerobic capacity in thermogenesis ($\dot{V}_{O_{2sum}}=5.46 \text{ ml O}_2 \text{ min}^{-1}$ vs $3.81 \text{ ml O}_2 \text{ min}^{-1}$). Interestingly, these changes in maximal aerobic power output were not reflected in mass-adjusted BMR, which showed no significant change with age (see also O'Connor and Buffenstein, 2000). Consequently, the factorial aerobic scope in exercise ($\dot{V}_{O_{2max}}/\text{BMR}$) declined from 6.4–6.5 in 100-day-old mice (means for males and females, respectively) to 4.9–5.8 in 1800-day-old animals, a reduction of 10–25%. Corresponding values for thermogenic scope were 7.2–7.3 (100 days) and 5.0–5.9 (1800 days), a decline of 19–31%.

There are surprisingly few reports of aerobic performance changes across the life span in small mammals, but there is an extensive literature on the effects of age on human performance, including aerobic capacity and athletic competition. These data serve as a useful frame of reference for our results with *Peromyscus*. As for deer mice, human aerobic capacity declines considerably with age: between the third and the seventh decade of life, the $\dot{V}_{O_{2max}}$ of both athletes and untrained males decreases by 35–45% (Goldberg et al., 1996). Similarly, maximal work rate during bicycle ergometry declines by approximately 1.3% annually (Stones and Kozma, 1985) or a 40% reduction over 40 years. Human athletic performance in long-duration races that are highly dependent on aerobic power output shows similar trends. For example, in distance running events (0.8–42 km), slopes of log_e-transformed race times on age are about 0.01 for men and 0.02

for women (Stones and Kozma, 1985), which equates to speed decreases of roughly 30–50% over 40 years. Comparable reductions occur in strength tests (e.g. a 30% decline in maximum handgrip force over 40 years; Stones and Kozma, 1985). When normalized to fractions of life span, the magnitude of age-related performance changes in humans (30–50% declines in several indices between the third and seventh decade) are somewhat greater than those seen in deer mice (22–30% reduction of aerobic capacity between 100 days and 1800 days).

As a second frame of reference, it is instructive to compare the impacts of age on aerobic performance with the magnitude of two other factors that are known to affect deer mouse aerobic physiology: phenotypic plasticity in temperature acclimation and genetic adaptation of hemoglobins to different altitudes. In *Peromyscus* and many other small rodents, exposure to a cold environment in the laboratory or the field induces large increases in thermogenic capacity (e.g. Rosenmann et al., 1975; Heimer and Morrison, 1978; Wickler, 1980). The $\dot{V}_{O_{2sum}}$ of deer mice held at 3–5°C for 3 months was 31% greater than that of controls held at 20–22°C (Hayes and Chappell, 1986), and in wild deer mice in California, winter acclimatization increased $\dot{V}_{O_{2sum}}$ by up to 50% (Hayes, 1989a,b). Thus, the impact of age (up to a 30% reduction in $\dot{V}_{O_{2sum}}$ in very old mice) is equivalent to a substantial fraction of the effects of temperature acclimation on thermogenic capacity.

Deer mice have a diverse array of α -globin polymorphisms that are geographically correlated with altitude (Snyder et al., 1988) and affect blood oxygen affinity (Chappell and Snyder, 1984). Studies of genetically standardized laboratory lines and wild-caught samples (comprised primarily of young animals) indicate that mice with the 'correct' hemoglobin for either low or high altitude (in these tests, 340 m vs 3800 m) have a performance advantage of approximately 10% in both $\dot{V}_{O_{2max}}$ and $\dot{V}_{O_{2sum}}$ (Chappell and Snyder, 1984). That difference –

which was proposed as a selective mechanism for the evolution of the hemoglobin polymorphisms – is considerably less than the potential effects of age on aerobic performance in these mice.

Body composition and performance changes

Decreases in age-related aerobic performance in mice older than 485 days could have several functional explanations. At the whole-animal level, they could result from a decline in the mass-specific power output capacity of central ‘supply’ organs (heart, lungs, etc.) or peripheral effectors (skeletal muscle or, for thermogenesis, brown adipose tissue). Declines with age in muscle fiber force generation per cross-sectional area in laboratory mice and rats (Thompson and Brown, 1999; Gonzalez et al., 2000) are consistent with this hypothesis. Alternatively, mass-specific activities of central and peripheral organs could be unchanged with age, but overall body composition (e.g. the proportions of fat vs lean tissue or the proportion of lean tissue allocated to different organs) could differ.

Our results with deer mice are most consistent with the first hypothesis (a decline in mass-specific lean tissue function with age). We found no consistent age-related changes in lean tissue mass, heart mass or lung mass in old mice (other than those in proportion to changing body mass; Table 4), indicating that the relative size of these organs did not decline as the mice grew older and whole-animal maximal power output decreased (in fact, the fraction of total body mass comprising lean tissue tended to increase with age; Table 4). In this respect, deer mice apparently differ from humans: age-related declines in human exercise performance are in large part due to reductions in proportional muscle mass (summarized in Stones and Kozma, 1985), although a number of other factors are also important (summarized in Goldberg et al., 1996).

Ecological and evolutionary considerations

In terms of behavior and ecology, the effects of 22–30% decreases in aerobic capacity are likely to be highly significant for old deer mice. Running speed in mammals is a linear function of metabolic power output (Taylor et al., 1970), so the maximum sustainable running speed of a 5-year-old deer mouse will be approximately 20% lower than that of an equal-sized young mouse. It is also likely that endurance at high exercise intensities will be concomitantly reduced in old animals. Although deer mice seem unlikely to depend on endurance performance for very critical behaviors (such as escaping from predators), sustainable locomotor capacity is an important component of their ecology. Wild deer mice in a high-altitude population in the White Mountains of eastern California (from which our colony originated) routinely travel hundreds of meters during nocturnal activity periods (Hayes, 1989a; Hayes and O’Connor, 1999), so a decrease in exercise capacity could have a significant impact on home range size and hence resource availability.

Age-related declines in thermogenic capacity may be even more important to deer mouse ecology. Previous calculations

from lab studies (Chappell and Snyder, 1984; Chappell and Holsclaw, 1984) and data on field metabolic rates (Hayes, 1989a,b) suggest that at typical nighttime temperatures at our White Mountains field site (approximately 0–5°C in summer and –5°C to –20°C in winter), deer mice are operating close to their maximal aerobic capacity. Therefore, the age-related decline we found in $\dot{V}_{O_{2sum}}$ would seriously constrain their capacity for activity at low temperatures. Consistent with that estimate, Hayes and O’Connor, (1999), working at the same site, found evidence of selection favoring high thermogenic \dot{V}_{O_2} in wild deer mice (survivorship was positively correlated to $\dot{V}_{O_{2sum}}$). An important caveat for these conclusions stems from lack of cold acclimatization in our test animals. As discussed above, long-term cold exposure induces large increases in $\dot{V}_{O_{2sum}}$ in deer mice. It is unknown if the age-related decline in $\dot{V}_{O_{2sum}}$ we found in warm-acclimated mice is also evident after cold acclimation, but the simplest assumption is that it is.

Despite their potential for substantial impacts on important ecological factors, it is doubtful that age-related aerobic performance declines have tangible evolutionary significance for deer mice. This seemingly contradictory conclusion stems from population age structure: most rodents (including deer mice) and other small mammals have very short mean life spans, even after attaining reproductive adulthood (e.g. Millar, 1989; Price and Kelly, 1994; Duquette and Millar, 1995). A life table is not available for deer mice from our White Mountain source population, but it is unlikely that more than a small fraction of wild deer mice attain the ‘breakpoint’ age of 485 days (Hayes and O’Connor, 1999). Even fewer continue to survive long enough to experience biologically meaningful declines in aerobic performance, and fewer still will have a chance to reproduce at such an advanced age. This demographic structure greatly reduces the power of selection to generate evolutionary change in heritable performance declines that appear only in old individuals (Williams, 1957; Hamilton, 1966; Charlesworth, 1980; Rose, 1991).

One final caveat is worthy of consideration. Due to the constraints of our ‘cross-sectional’ experimental design, the youngest animals in our data set were the products of several more laboratory generations (up to 5) than the oldest individuals (generation 2). Even without intentional artificial selection, captive breeding often results in evolutionary adaptation to culture conditions (i.e. domestication), so it is conceivable that our results show genetic change between generations instead of the effects of age. That seems unlikely, since the direction of change – higher performance in younger animals from later laboratory generations – is opposite to what is expected from genetic adaptation to relatively benign laboratory conditions (a decline in performance with domestication; e.g. Swallow et al., 1998). Laboratory rearing appears to reduce the aerobic performance of deer mice: wild-caught individuals had higher performance than lab-bred mice even after the latter were acclimated to local altitude and temperature (Chappell and Snyder, 1984; Hayes, 1989a,b; Hammond et al., 2002).

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