

Relationships among running performance, aerobic physiology, and organ mass in male Mongolian gerbils

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Running title: Exercise performance in gerbils

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Summary

Relationships among individual variation in exercise capacity, resting metabolism, and morphology may offer insights into the mechanistic basis of whole-animal performance, including possible performance trade-offs (e.g., burst versus sustainable exercise; resting 'maintenance' costs versus maximal power output). Although several studies of correlations between performance, metabolism, and morphology have been performed in fish, birds and squamate reptiles, relatively little work has been done with mammals. We measured several aspects of forced and voluntary locomotor performance in Mongolian gerbils (*Meriones unguiculatus*), along with minimal and maximal aerobic metabolic rates and organ sizes (mainly visceral organs and the musculoskeletal system). Maximal sprint and aerobic speeds and maximal oxygen consumption ($\dot{V}O_2\text{max}$) during forced exercise were similar to those of other small rodents; basal metabolic rate was below allometric predictions. At all tested speeds, voluntary running had a lower energy cost than forced treadmill running, due primarily to a higher zero-speed intercept of the speed-versus-power (oxygen consumption) relationship during forced running. Incremental costs of transport (slopes of speed-versus-power regressions) were slightly higher during voluntary exercise. Few of the correlations among performance variables, or between performance and organ morphology, were statistically significant. These results are consistent with many other studies that found weak correlations between organismal performance (e.g., $\dot{V}O_2\text{max}$) and putatively relevant subordinate traits, thus supporting the idea that some components within a functional system may exhibit excess capacity at various points in the evolutionary history of a population, while others constitute limiting factors.

Key words: energetics, individual variation, locomotion, maximum oxygen consumption, *Meriones unguiculatus*, metabolic rate, rodent, symmorphosis

Introduction

Mechanistic, comparative, ecological, and evolutionary physiologists have long been interested in animal locomotion (e.g., Irschick and Garland, 2001; Oufiero and Garland, 2007). In most non-sessile animals, locomotor performance can be related intuitively, and sometimes empirically, to such components of Darwinian fitness as escape from predators, prey capture, foraging, courtship, territorial behavior, combat or migration (e.g., Sinervo et al., 2000; Perry et al., 2004; Husak, 2006). From a mechanistic perspective, locomotion is perhaps the most integrative (Dickinson et al., 2000) and demanding aspect of organismal physiology, as it is dependent on coordinated functioning of numerous organ systems and often requires the highest attainable intensities of aerobic and anaerobic power output. In an ecological context, locomotor costs are an unavoidable part of an animal's energy budget, and hence impact food requirements, foraging efficiency, and allocation of energy among competing demands of maintenance, growth, and reproduction.

Decades of comparative work have yielded a broad understanding of energetics and biomechanics during terrestrial locomotion, swimming, and flying (e.g., Taylor et al., 1970; Tucker, 1975; Miles, 1994; Wainwright et al., 2002; Alexander, 2003; Bejan and Marden, 2006). The mass scaling of locomotor costs has been documented extensively, as has the magnitude of interspecific variation in performance abilities during burst and sustainable exercise (e.g., Djawdan and Garland, 1988; Garland et al., 1988; Djawdan, 1993; Domenici and Blake, 1997; Bonine and Garland, 1999; Weibel et al., 2004). A number of comparative studies have also explored the mechanistic underpinnings of locomotor performance; perhaps the best known of these is the classic series of papers from C. R. Taylor, E. Weibel, and their colleagues on the scaling of mammalian oxygen uptake, transport and delivery systems in relationship to aerobic capacity in running exercise (e.g., Weibel and Taylor, 1981; Weibel, 1984; Weibel et al., 2001; 2004).

More recently, an important contribution of evolutionary physiology has been a growing focus on intraspecific studies (Bennett, 1987; Garland and Carter, 1994), with one emphasis being the exploitation of individual variation to gain insights into performance across many levels of integration. This approach has been used to examine trade-offs between burst versus endurance performance, links between resting and maximal metabolic rates, interactions between aerobic capacity and running speed or endurance, and the sub-organismal traits (limb dimensions, organ size, enzyme function, mitochondrial properties, etc.) that 'drive' performance variation and hence might be expected to change in response to training (phenotypic plasticity) and/or in response to selection (genetic evolution). A number of such studies (e.g., Garland, 1984; Garland and Else, 1987; Gleeson and Harrison, 1988; Chappell and Bachman 1995; Hammond et al., 2000; Sinervo et al., 2000; Vanhooydonck et al., 2001; Harris and Steudel, 2002; Odell et al., 2003; Pasi and Carrier, 2003; Brandt and Allen, 2004; Kemp et al., 2005) have found an assortment of within-species associations between traits, but the combined results reveal surprisingly few consistent overall patterns (see Discussion).

Here we report results of a comprehensive intraspecific study of locomotor performance, aerobic physiology, and organ size in Mongolian gerbils (*Meriones unguiculatus*: Milne-Edwards 1867). Mongolian gerbils are small, quadrupedal rodents native to open grasslands and sandy deserts in central Asia, sheltering in burrows but foraging and performing other activities above ground (Naumov and Lobachev, 1975; Ågren et al., 1989). They show no obvious morphological specialization for sprinting, distance running, or digging and appear to be locomotor generalists. Although domesticated, gerbils have been removed from the wild state for far fewer generations than laboratory mice or rats: they were first brought into laboratory culture in 1954 (Schwentker, 1963).

Our study took advantage of a recently developed method for obtaining detailed information on the energetics and behavior of voluntary running, in addition to more traditional tests of the limits to performance in forced exercise. As well as providing data on the intermediate work intensities frequently used by animals, this approach might indicate if locomotor physiology differs between forced and voluntary running (Chappell et al. 2004; Rezende et al. 2006), and if routine voluntary activity is constrained by physiological limits. Additionally, we were interested in interactions between different performance traits: sprint versus aerobic performance, basal versus maximal aerobic metabolism, and relationships between aerobic physiology and voluntary running. Finally, to explore potential morphological bases for performance capacity, we examined size variation in major organ systems, including central support organs (heart, lung, digestive tract, liver, kidneys), control systems (brain), and the primary peripheral effector of locomotion, the musculoskeletal system.

Methods

Animals: We obtained gerbils from a breeding colony at the University of California, Riverside; the founding stock came from Harlan Sprague-Dawley, Indianapolis, Indiana. To avoid potential complications of estrous cycles, we used only adult males that were between 92 and 174 days old at the conclusion of measurements (mean 123 days, SD 22 days; N = 40). Gerbils were housed initially in standard polycarbonate cages (48 X 27 X 20 cm) in groups of 2-5 age-matched males; during experiments they were housed singly. The light cycle was 12 h L: 12 h D (lights on at 0700 – 1900 h), temperature in the animal room was maintained at ~ 23 °C, and animals had *ad libitum* access to water and commercial food (Purina Rodent Chow 5001), supplemented periodically with sunflower seeds, oats, and carrots (Saltzman et al., 2006).

We collected data from each animal on the following schedule: voluntary wheel running (acclimation, days 1-4; measurements, days 5-6), maximal oxygen consumption

during forced treadmill exercise ($\dot{V}O_{2\max}$; days 7 and 8), metabolic costs of transport on a treadmill (day 9), maximal sprint speed (days 10 and 11), basal metabolic rate (BMR) (night of day 11), and then sacrifice for organ mass measurements (day 12).

All animal procedures were approved by the U.C. Riverside Institutional Animal Care and Use Committee and are in compliance with U.S. National Institutes of Health Guidelines (NIH publication 78-23) and U.S. laws.

Energetics of voluntary activity: We used enclosed running wheel respirometers that permitted simultaneous measurement of wheel speed and gas exchange every 1.5 sec for 48 h, as described in Chappell et al. (2004; see also Rezende et al., 2006). The wheels (Lafayette Instruments, Lafayette, Indiana, USA) were constructed of stainless steel and Plexiglas, with a circumference of 1.12 m. Gerbils were allowed 4 days access to similar but unenclosed wheels to acclimate prior to measurements. Each Plexiglas wheel enclosure had an internal fan to rapidly circulate and mix air and contained a standard polycarbonate mouse cage (27.5 cm X 17 cm X 12 cm, L X W X H) with bedding, a drinking tube, and a food hopper containing rodent chow. Gerbils could move freely between the cage and the wheel through a 7.7 cm diameter port cut into the wall of the cage. Enclosures were supplied with dry air at flow rates of 2500 ml/min STP ($\pm 1\%$) by Porter Instruments mass flow controllers (Hatfield, Pennsylvania, USA). The speed and direction of wheel rotation were transduced by a small generator that functioned as a tachometer.

Output ports directed air from enclosures to oxygen and CO₂ analyzers ('Oxilla' and CA-2A, respectively; Sable Systems, Henderson, Nevada, USA), which subsampled excurrent air at about 100 ml/min. Subsampled air was dried with magnesium perchlorate prior to analysis. A computer-controlled solenoid system obtained 3-min reference readings (dry air) every 42 min. Data from all instruments were recorded by a Macintosh computer equipped with an analog-to-digital converter and Warthog

Systems 'LabHelper' software (www.warthog.ucr.edu). Because of the large chamber volume we smoothed metabolic data to minimize electrical noise and used the 'instantaneous' transformation to accurately resolve short-term events (Bartholomew et al., 1981). The effective volume, computed from washout curves, was 17 L. Wheel measurements lasted approximately 47.5 h. 'LabAnalyst' software (Warthog Systems) was used to smooth data, subtract baseline values, correct for lag time (i.e., synchronize wheel speed with gas exchange), replace reference data by interpolation, compute $\dot{V}O_2$ and $\dot{V}CO_2$, and extract the following values:

- Average daily metabolic rate (ADMR; ml O₂/min)
- respiratory exchange ratio (RER; $\dot{V}CO_2/\dot{V}O_2$; 24 h mean)
- minimum resting $\dot{V}O_2$ over 10 min (resting metabolic rate, RMR)
- maximum voluntary $\dot{V}O_2$ over 1, 2, and 5 min ($\dot{V}O_{2v1}$, $\dot{V}O_{2v2}$, $\dot{V}O_{2v5}$)
- maximum instantaneous wheel speed (V_{max}) over a 1.5 sec interval
- maximum wheel speed over 1, 2, and 5 min (V_{max1}, V_{max2}, V_{max5})
- total distance run (D_{run}) and total time run (T_{run}); 24 h means

We used a stepped sampling procedure, with 1-minute averages separated by 3 minutes, to obtain measures of $\dot{V}O_2$, $\dot{V}CO_2$, and running speed without autocorrelation (successive measurements over short intervals are not independent, because wheel speed and metabolism do not respond instantly to changes in behavior). With this protocol there is no statistically significant correlation between sequential 1-minute averages (Chappell et al., 2004; Rezende et al., 2005; 2006). Previous studies with this system used 5-min minimum averages for RMR, but in the present study we noted that although the 5-min average RMR was only 9% lower than 10-min average RMR, CVs were about 50% greater for the shorter averaging interval.

Maximal oxygen consumption: We used a motorized treadmill inclined at 19° above horizontal to elicit $\dot{V}O_{2max}$ (Kemi et al., 2002). Gerbils were placed in a Plexiglas

running chamber (the working section was 33 cm long, 12.5 cm wide, and 12 cm high) that slid above the moving tread, with the bottom edge sealed with felt strips and low-friction Teflon tape. The chamber was supplied with air under positive pressure (8700 ml/min STP from a mass flow controller) through six input ports spaced along the top of the chamber. About 1000 ml/min of air was pumped out through four ports on the sides; the remainder escaped under the bottom edge of the chamber. About 150 ml/min of excurrent air was dried with magnesium perchlorate, flowed through a CO₂ analyzer (LiCor 6251; Lincoln, Nebraska, USA), scrubbed of CO₂ and redried (soda lime and Dryerite, respectively), and passed through an O₂ analyzer (Applied Electrochemistry S-3A; Pittsburgh, Pennsylvania, USA). Flow rates, tread speed, and gas concentrations were recorded every 1.0 sec by a computer, using 'LabHelper' software. As with the running-wheel chamber, we used the 'instantaneous' correction (Bartholomew et al., 1981) to accurately resolve short-term events. The effective volume of the running chamber was 7200 ml.

An electrical stimulation grid at the rear of the chamber delivered 30-50 VAC through a 10 K- Ω resistor to provide motivation (Friedman et al., 1991; Swallow et al., 1998; Dohm et al., 2001). We gave gerbils several minutes to acclimate to the chamber before starting the tread and accelerating over several seconds to low speed (1-1.5 km/h), which was maintained for about 30 sec. Most individuals quickly oriented correctly and ran well. Subsequently we increased speed every 30 sec in steps of about 0.4 km/h until the animal could no longer maintain position on the tread, or until VO₂ did not increase with increasing speed, or until the gerbil touched the shock grid for more than 2 sec. Runs lasted 2.5 to 8 min; all animals attained $\dot{V}O_{2\max}$ at speeds less than the maximum tread speed of about 3.9 km/h.

Metabolic costs of transport: We used the same motorized treadmill, flow rates, and sample rates to measure energy metabolism during sustained running, but the treadmill was level instead of inclined. Gerbils were tested at speeds of 0.6 km/h to 3.8 km/h, in

increments of about 0.5 km/h. Speeds were presented in random order, and we attempted to obtain 10 min of steady running at each speed. Some animals failed to run steadily at some speeds, especially the lowest and highest speeds, but most individuals performed well across a substantial speed range. Usually, gerbils were rested for at least 20 min between speeds; they always resumed exploratory behavior within 5 min after the end of a running bout (often, immediately after the tread was stopped). Because steadily running animals usually adapted quickly to speed changes (more rapidly than if they were accelerated from rest), we often made measurements at two speeds without an intervening rest period. Reference readings of O₂ and CO₂ content were obtained immediately before and after each running bout.

Basal metabolic rate: Captive Mongolian gerbils do not have a strong circadian activity cycle and exhibit activity during both night and day (Lerwill, 1974; Sun and Jing, 1984). We measured BMR at night. At approximately 17:00 h, following a 4-6 h fast, animals were placed in 1.5 L Plexiglas metabolism chambers supplied with air at 620 ml/min STP. The chambers were held at 30 ± 0.3 °C (well within the species' thermal neutral zone of 26-38 °C; Wang et al., 2000) in an environmental cabinet. About 100 ml/min of excurrent air was scrubbed of CO₂ and dried, then passed through a two-channel Applied Electrochemistry S-3A/2 oxygen analyzer that allowed simultaneous measurements on two animals. Flow rates, temperature, and oxygen concentration were recorded every 4 sec, and a computer-controlled solenoid obtained 3-min reference readings every 42 min until animals were removed at approximately 08:00 h the following morning. Accordingly, the duration of fasting was at least 19 h at the end of measurements. We used the lowest 10-min continuous average $\dot{V}O_2$ to represent BMR (see above).

Gas exchange calculations: In all respirometry systems, mass flow controllers were upstream of metabolism chambers and air was supplied under positive pressure. Nevertheless, differences in plumbing and gas handling necessitated use of different equations to compute $\dot{V}O_2$ for treadmill tests and BMR measurements, and during voluntary exercise. For treadmill tests and BMR, we absorbed CO_2 prior to O_2 measurements and calculated $\dot{V}O_2$ as:

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

where \dot{V} is flow rate (ml/min STP) and F_iO_2 and F_eO_2 are the fractional O_2 concentrations in incurrent and excurrent air, respectively (F_iO_2 was 0.2095 and F_eO_2 was always > 0.205). For voluntary exercise, we did not remove CO_2 as required for Eqn. 1 (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:

$$\dot{V}O_2 \text{ (ml/min)} = \dot{V} \cdot (F_iO_2 - F_eO_2) / (1 - F_eO_2 \cdot (1 - RQ)) \quad (2)$$

where RQ is the respiratory quotient ($\dot{V}CO_2/\dot{V}O_2$). Based on preliminary data and previous measurements (Chappell et al. 2004), we used a constant RQ of 0.85. Use of 0.85 creates a 3% overestimate of $\dot{V}O_2$ if the real RQ = 1.0 and a 2% underestimate of $\dot{V}O_2$ if the real RQ = 0.7. We selected a conversion equation based on constant RQ instead of using measured CO_2 concentration in $\dot{V}O_2$ calculations in order to minimize potential errors caused by unequal response times of O_2 and CO_2 analyzers. This was particularly important in our system because behavior and metabolism changed rapidly and instantaneous conversions (Bartholomew et al., 1981) were necessary.

For the same reasons we also assumed a constant RQ of 0.85 to calculate $\dot{V}CO_2$ for both voluntary exercise and treadmill tests:

$$\dot{V}CO_2 \text{ (ml/min)} = \dot{V} \cdot (F_eCO_2 - F_iCO_2) / (1 - F_eCO_2 \cdot (1 - (1/RQ))) \quad (3)$$

where F_iCO_2 and F_eCO_2 are the incurrent and excurrent fractional concentrations of CO_2 , respectively. Given that F_eCO_2 was always < 0.0025 , the value of RQ had very

little effect on calculated $\dot{V}CO_2$ (the maximum error for real RQs between 0.7 and 1.0 was 0.2%).

Gas exchange validations and energy equivalence:

All mass flow controllers used in the study (for measurements of BMR, voluntary exercise, and treadmill running) were calibrated against the same dry volume meter (Singer DTM-115; American Meter Company, Horsham, PA). Once per week, CO_2 analyzers were zeroed with room air scrubbed of CO_2 (soda lime) and spanned against a precision gas mixture (0.296% CO_2 in air). Drift between calibrations was small (<1% of the span gas concentration).

The wheel chambers used for voluntary running measurements were calibrated using a nitrogen dilution procedure (Fedak et al. 1981). Briefly, we added a small, precisely measured flow of hypoxic gas (180 ml/min, 14.25% O_2 , balance N_2) to a flow of 2300 ml/min of air through the chamber. The hypoxic gas was released at specific locations within the chamber via a thin, flexible tube inserted through the airtight port for the drinking tube. The depletion in O_2 content relative to pure air was equivalent to a $\dot{V}O_2$ of 12.06 ml/min. We recorded excurrent gas concentrations and calculated $\dot{V}O_2$ with the same procedures used for animals. A series of such tests with hypoxic gas released in different locations (including inside the running wheel itself and in the extreme corners of the mouse cage) yielded $\dot{V}O_2$ measurements that were always within 2.4% of the 'real' value of 12.06 ml/min, with a mean of 12.02 ml/min.

A similar procedure was used for the treadmill system. Experiments with a 100% N_2 gas point source revealed no position effects (a constant low flow rate of N_2 through a small-diameter tube yielded equal deflections in O_2 concentration at all positions used by gerbils within the chamber, at tread speeds typical of gerbil running). Calculated $\dot{V}O_2$ measurements were within 2-3% of values expected from flow rates of air and N_2 (Fedak et al. 1981).

We converted rates of oxygen consumption to rates of energy expenditure by multiplying $\dot{V}O_2$ by 20.1 J/ml O_2 , which is appropriate for a mixed diet (Schmidt-Nielsen, 1997).

Sprint speed: Maximum sprint velocities (speeds that gerbils could sustain for at least 2 sec) were measured on a 1.4 m long, high-speed treadmill (Bonine and Garland, 1999). A digital readout displayed treadmill velocity with a resolution of ± 0.03 km/h over the speed range used by gerbils (up to 14.5 km/h). A gerbil was placed in a 12 cm-wide channel formed by plastic walls suspended a few mm above the tread. When the animal faced forward, the belt was started and rapidly accelerated for as long as the animal matched its speed. Forward running was encouraged by the operator's gloved hand, and the trial was terminated when the animal no longer maintained position. Runs lasted less than 1 minute. The highest attained velocity was read from the digital readout, and a qualitative score of running performance was assigned. Data from animals that refused to run were not used in analyses (see Results). Gerbils were tested twice, once on each of two successive days, and each individual's highest speed on either day was used as its maximum sprint speed.

Morphology: Within 24 h of the end of BMR measurements, animals were euthanized (CO_2 inhalation), weighed, measured (snout-rump length, head length from nose to the rear of the skull, head width at the ears, and hind foot lengths), and dissected. We removed the brain, ventricles of the heart, lungs, liver, spleen, kidneys, stomach, small intestine, large intestine, caecum, and testes. The ventricles were blotted to remove blood, and the contents of the digestive tract were removed. The vas deferens, epididymis, prostate, and seminal vesicles were collectively weighed and referred to as 'other reproductive structures'. Organs were trimmed of fat, rinsed in physiological saline, blotted dry, and weighed (± 0.0001 g; Denver Instruments XE-100; Denver,

Colorado). We removed and weighed the gastrocnemius muscles, and the remaining musculoskeletal system (all skeletal muscles and bones except the head, tail, and feet) was trimmed of fat and weighed. Organs were then dried to constant mass at 50 °C and re-weighed.

Statistics. Because organ size, aerobic physiology, and locomotor performance are influenced by body size and potentially by age, we included body mass and age (in days) as covariates, or computed residuals from regressions on mass and age. Metabolic and body mass data were \log_{10} -transformed prior to analysis; results are presented in untransformed units (as mean \pm SD unless otherwise noted). The significance level α was 0.05 (2-tailed tests). Multiple simultaneous tests (such as in large correlation tables) are at risk of inflated Type 1 error rates. To compensate, we used two methods. First, we provide an adjusted α from a sequential Bonferroni correction (Rice, 1989). Such corrections have been criticized as inappropriately conservative (they may increase type II errors unacceptably, e.g. Nakagawa, 2004), so we also used the q-value procedure developed to control false discovery rates (FDR; Storey and Tibshirani, 2003; Storey, 2003). Values of π_0 (the overall proportion of true null hypotheses) and corresponding q-values were generated with the 'Qvalue' library run in the R statistical package (The R Foundation for Statistical Computing) using the 'Bootstrap' option. Other analyses were performed using the t-test, regression and GLM procedures in SPSS for the Macintosh (SPSS, Incorporated, Chicago, Illinois).

Results

Mean body mass of the 40 adult male gerbils was 67.7 ± 6.0 g (Table 1). Age at sacrifice ranged from 92 to 174 days (mean 123 ± 22 days). The correlation between age and body mass was marginally significant (mass = $57.6 + 0.082 * \text{age}$, $r^2 = .095$, $F_{1,38} = 4.0$, $P = .053$). Some individuals were not tested for all measured traits and a few

refused to perform in certain procedures (mainly sprinting or treadmill cost of transport measurements).

Most of the measured traits, including both performance and morphological measures, varied significantly with body mass, and several varied significantly with age (Table 2). To compare the relative variability of different traits, we used residuals from allometric equations (Garland, 1984). For variables that do not scale with mass or age, the SD of residuals (from \log_e -transformed data) is approximately equivalent to the CV of untransformed data. For variables that show significant scaling, the SD of residuals (from \log_e -transformed data) is equivalent to the CV of untransformed data after removing variation related to age and mass (Lande, 1976; Garland, 1984). In our data set, CV ranged from 2-3% for brain and musculoskeletal system to about 50% for voluntary wheel-running times and distances (Table 2).

Basal and maximal metabolic rate: Because of equipment constraints at the beginning of the study, not all animals could be tested for BMR, and a few gerbils did not attain low and stable $\dot{V}O_2$ during BMR measurements. BMR was independent of age but was positively correlated with body mass, as would be expected (Table 2; BMR in ml O_2 /min = $.00729 * \text{mass}^{1.18}$; $r^2 = .338$, $F_{2,26} = 12.0$, $P = .0019$; Fig. 1). For a gerbil of average mass (68.4 g in the 27 animals tested for BMR), the predicted BMR was 1.07 ml O_2 /min (1.05 ml O_2 /min for the average mass of 67.7 g for all 40 gerbils in the study).

Maximal oxygen consumption during forced treadmill exercise ($\dot{V}O_{2\text{max}}$) was significantly correlated with both body mass and age, scaling positively with mass and slightly negatively with age (Table 2). For a gerbil of the average age and mass in this study, predicted $\dot{V}O_{2\text{max}}$ was 11.2 ml O_2 /min, and factorial aerobic scope ($\dot{V}O_{2\text{max}} \div \text{BMR}$) was 10.7. Measured aerobic scopes ($N = 27$) ranged from 5.29 to 15.2, averaging 10.1 ± 2.48 .

RER at $\dot{V}O_{2\max}$ averaged 0.975 ± 0.072 (range .83 -1.14) and was independent of age and mass ($P > .55$ for both). However, RER was negatively correlated with $\dot{V}O_{2\max}$ ($F_{1,38} = 6.4$, $P = .015$), declining from a predicted 1.07 in a gerbil with $\dot{V}O_{2\max} = 7$ ml O_2 /min to 0.96 in a gerbil with $\dot{V}O_{2\max} = 12$ ml O_2 /min. We did not measure $\dot{V}CO_2$ during BMR studies.

Sprint performance: Some gerbils refused to run on the high-speed treadmill or ran poorly on one or both of the two days of testing. For 16 individuals with acceptable tests on both days, speed declined by 17%, on average, from day 1 to day 2 (11.5 ± 2.02 and 9.57 ± 2.08 km/h, respectively; paired t-test; $P = 0.0018$). However, individual performances were significantly repeatable between days, as indicated by Pearson's $r = .506$ ($F_{1,14} = 4.82$, 2-tailed $P = .045$) (Nespolo and Franco, 2007). For individuals that performed acceptably on at least one day ($N = 34$, mean mass 68.2 ± 6.1 g), we used the highest attained speed from either day ('sprint speed') in other analyses. Age and body mass did not affect sprint speed (Table 2), and the mean maximum sprint speed was 10.8 ± 2.0 km/h (Table 1).

Behavior and metabolism during voluntary activity: Gerbils did not make extensive use of the running wheels. Daily averages were 1.24 km and 83.3 min (Table 1), which yields a mean running speed of 0.89 km/h. Neither body mass nor age predicted either distance run or time spent running (Table 2). The majority of time spent running was at low speeds (< 0.5 km/h, see Fig. 2a), but most of the distance covered during running was at speeds between 0.5 and 1.5 km/h (Fig. 2b). Maximum voluntary speeds averaged over 1, 2, and 5 min were tightly correlated (Fig. 3a; regressions forced through the origin), with V_{\max_2} averaging 83% of V_{\max_1} ($r^2 = .993$) and V_{\max_5} averaging 67% of V_{\max_1} ($r^2 = .985$).

Average daily metabolic rates (ADMR; kJ/day) were strongly positively correlated with body mass but independent of age (Table 2), and averaged about 1.6 X BMR (Fig. 1). Minimum resting (non-fasted) metabolic rates in the wheels (RMR) were slightly but significantly lower than BMR (0.945 ± 0.129 versus $1.07 \pm .206$ ml O₂/min, respectively; 2-tailed P = .0108, paired t-test; mean RMR mass 67.7 g; mean BMR mass 68.4 g).

The maximal voluntary $\dot{V}O_2$ was always much lower than the $\dot{V}O_{2max}$ elicited during forced treadmill exercise (Fig. 1). For 1-min averages, maximal voluntary $\dot{V}O_2$ ($\dot{V}O_{2v1}$) was 45% of $\dot{V}O_{2max}$ (5.1 versus 11.3 ml/min, respectively; P < .0001, paired t-test). Similar to the results for maximum voluntary speeds averaged across different intervals, voluntary $\dot{V}O_2$ averaged over 2- and 5- min intervals was tightly correlated with $\dot{V}O_{2v1}$ (Fig. 3b; $r^2 = .999$ and $.994$, respectively), but slightly lower : $\dot{V}O_{2v2}$ was 96% of $\dot{V}O_{2v1}$, and $\dot{V}O_{2v5}$ was 87% of $\dot{V}O_{2v1}$ (regressions forced through the origin).

Respiratory exchange ratios averaged over 24 h were independent of mass but slightly negatively correlated with age ($F_{1,39} = 7.2$, $r^2 = .16$, P = .011), declining from .96 at 90 days to .87 at 170 days. These values are consistent with the RER of .92 expected from steady-state complete oxidation of the diet (caloric content: 59.4% carbohydrate, 28.4% protein, and 12.3% fat according to the manufacturer).

Forced and voluntary locomotor costs: Most gerbils performed sufficiently well during forced treadmill locomotion and voluntary running to provide useable data on metabolic costs of locomotion (statistically significant regressions of metabolic rate on speed; N = 35 for forced exercise, N = 38 for voluntary exercise, N = 34 for both forced and voluntary exercise). Gerbils used roughly comparable speed ranges in both conditions, although mean speeds were considerably lower in voluntary exercise (2.18 ± 1.0 km/h in forced exercise vs. 0.73 ± 0.78 km/h in voluntary exercise, $F_{1,3708} = 818$, P < .0001; Fig. 4). In forced exercise, the minimum treadmill speed was 0.6 km/h

and the maximum speed was about 3.8 km/h. During voluntary exercise, animals regularly used speeds lower than 0.6 km/h. The mean maximum instantaneous speed (1.5-sec average) was 4.1 km/h and the mean highest 1-min average speed was 2.5 km/h.

Body mass was significantly positively correlated with $\dot{V}O_2$ during both forced and voluntary running (Table 2), but conversion of $\dot{V}O_2$ to mass-specific power output ($\text{kJ kg}^{-1} \text{h}^{-1}$) eliminated the statistical significance of body mass (results not shown). There was little overlap in metabolic costs of forced and voluntary running, either in individuals or for pooled data (Fig. 4), despite fairly similar ambient temperatures (22-24 °C for forced exercise; 24-28 °C for voluntary exercise). To avoid the confounding influence of dissimilar numbers of data points among animals, particularly for voluntary running, we calculated slopes and intercepts of the speed versus $\dot{V}O_2$ regression for each individual and used these in most analyses. These regressions describe the cost of transport (COT), and we refer to COT in treadmill exercise and voluntary exercise as tCOT and vCOT, respectively.

Because aerobic metabolism might be expected to plateau as animals approach their maximum aerobic speed, we used quadratic regressions to test for nonlinearity. Linear components of quadratic regressions were significant for all animals during forced exercise and for 32 of 38 individuals during voluntary locomotion. The quadratic component was never statistically significant during forced exercise, but was significant ($P < .05$) in 11 gerbils during voluntary locomotion, with a coefficient of -6.23 ± 6.55 (mean \pm SD; all significant quadratic coefficients were negative). Values of r^2 were only slightly higher for quadratic than for linear regressions ($.539 \pm .140$ versus $.525 \pm .130$ for voluntary running; $.874 \pm .106$ versus $.815 \pm .116$ for forced exercise). Because speed-versus-power relations for most individuals -- even in voluntary exercise -- did not have significant quadratic components, we used slopes and intercepts from linear regressions for subsequent analyses.

Intercepts were independent of body mass and age (Table 2) and differed significantly between forced and voluntary running ($t_{33} = 11.6$, $P < .0001$; paired t-test). The intercept for forced running ($75.8 \pm 15.7 \text{ kJ kg}^{-1} \text{ h}^{-1}$) was almost twice that for voluntary exercise ($38.9 \pm 3.4 \text{ kJ kg}^{-1} \text{ h}^{-1}$; Fig. 4). Body mass had a small but statistically significant effect on the slope for forced running, but not for voluntary exercise (Table 2). Age was unrelated to slope for both forced and voluntary running. Mean slope (the incremental cost of transport, or COT_{INC}) was slightly higher during voluntary running ($19.5 \pm 3.9 \text{ kJ kg}^{-1} \text{ km}^{-1}$) than during forced exercise ($15.7 \pm 7.2 \text{ kJ kg}^{-1} \text{ km}^{-1}$; $t_{33} = 3.34$, $P = .0022$; paired t-test). Using mean values of slopes and intercepts, at 4.0 km/h, the predicted power output was 18.5% higher for forced exercise ($138.6 \text{ kJ kg}^{-1} \text{ h}^{-1}$) than for voluntary exercise ($116.9 \text{ kJ kg}^{-1} \text{ h}^{-1}$), as was the total cost of transport (COT; $34.65 \text{ kJ kg}^{-1} \text{ km}^{-1}$ versus $29.23 \text{ kJ kg}^{-1} \text{ km}^{-1}$, respectively; Fig. 4).

We estimated maximal aerobic speed (MAS, the highest speed sustainable with aerobic power production) from treadmill-elicited $\dot{V}\text{O}_{2\text{max}}$ and the tCOT and vCOT slopes and intercepts. We assumed that MAS was the velocity at which the speed-versus- $\dot{V}\text{O}_2$ regression attained $\dot{V}\text{O}_{2\text{max}}$; hence, $\text{MAS} = (\dot{V}\text{O}_{2\text{max}} - \text{intercept})/\text{slope}$. We excluded unrealistically high forced-exercise MAS estimates for two individuals (MAS > 15 km/h, much faster than maximum sprint speed). Despite differences in slopes and intercepts, tCOT and vCOT converge at high running speeds (forced exercise has a higher intercept but lower slope than voluntary running). Estimated MAS did not differ significantly for forced and voluntary locomotion, averaging $8.03 \pm 1.28 \text{ km/h}$ ($N = 31$, mean mass = $68.4 \pm 6.2 \text{ g}$) in voluntary exercise and $7.82 \pm 1.43 \text{ km/h}$ ($N = 34$, mean mass = 68.3 ± 6.2) in forced exercise ($P = .605$, paired t-test). Therefore, the minimum cost of transport, which occurs at the highest aerobic speed (Taylor et al., 1982), did not differ between forced and voluntary running, although at lower speeds, absolute COT was lower in voluntary exercise than in forced exercise.

Relationships among metabolic and locomotor variables: Tests of interactions among metabolic, locomotor, and morphological traits were based on multiple simultaneous comparisons (Tables 3, 4, 5). Results are discussed in terms of the unadjusted α of .05, and after corrections for Type I errors via Bonferroni and FDR procedures; the P value distributions used to compute FDR are shown in Fig. 5.

Relationships among metabolic and locomotor performance variables (Table 3) were sometimes intuitive, but often not. BMR was not significantly correlated with any other metabolic or locomotor performance variable, including $\dot{V}O_{2\max}$. Estimates of maximal aerobic running speeds (vMAS and tMAS) were correlated with COT slopes and intercepts, and with $\dot{V}O_{2\max}$ (all of which were used to compute MAS), but $\dot{V}O_{2\max}$ was not correlated with other variables. As expected, the distance covered and time spent in voluntary running were tightly correlated, and both were positively correlated with ADMR. We found no statistical relationship between sprint speed and any metabolic trait, but sprint speed was positively correlated with maximum voluntary running speed and the intercept for voluntary running (vCOTint). Correlations between BMR and aerobic scope, $\dot{V}O_{2\max}$ and MAS, Vmax and distance, distance and run time, COT slopes and intercepts, and incremental COT and MAS remained significant after applying a q-value correction, and several remained significant even with the conservative Bonferroni correction.

Morphology and performance: We found few significant relationships between organ sizes and metabolic or locomotor performance (Table 4). Several behavioral and metabolic variables - running distance and time, COT slope and intercept in voluntary running, maximum voluntary running speed, and ADMR - were statistically independent of all morphological traits. Only one organ mass (caecum) was correlated with $\dot{V}O_{2\max}$. The highest voluntary $\dot{V}O_2$ ($\dot{V}O_{2v1}$) was negatively correlated with stomach and small intestine mass, but positively correlated with brain mass. Head dimensions and snout-

rump length were not correlated with performance limits (BMR, $\dot{V}O_{2\max}$, sprint speed). BMR was negatively correlated with the mass of the testes, but not with any other organ. The size of the musculoskeletal system was positively correlated with tCOT, but was not correlated with any other performance or metabolic variable. Gastrocnemius mass was not correlated with any performance or metabolic trait. After we applied a Bonferroni or q-value correction, none of the correlations retained significance.

Summed organ mass (including visceral organs, testes and other reproductive structures, and brain) was not correlated with any locomotor or metabolic variable.

Correlations among morphological traits: The measured organs (wet mass) totaled 59.3 ± 3.5 % of body mass, with the musculoskeletal system comprising 45.1 ± 3.0 % of body mass and the combined visceral organs, reproductive tissues, and brain comprising 14.3 ± 1.0 % of body mass. As fresh body mass included the contents of the digestive tract (unmeasured, but probably several g for some individuals), the fractions of body mass exclusive of digesta were somewhat higher than reported above.

Wet and dry organ masses were always highly correlated ($r^2 = .63$ -.94; $F > 65$ and $P < .0001$ in all cases). Consequently, relations among morphological traits were qualitatively similar for wet and dry masses (Table 5); we discuss dry mass results here. We found significant positive correlations among several visceral organs, notably those involved with food, nutrient, and metabolic waste distribution and processing (heart, liver, stomach, small intestine, large intestine, and kidney). The mass of the musculoskeletal system was positively correlated with heart, liver, and kidney mass. Testis mass was positively correlated with lung, liver, kidney, and spleen mass, but was independent of the mass of other reproductive structures. Snout-rump length was significantly related to head dimensions and the size of several visceral organs. Brain mass, gastrocnemius mass, and hind foot length were not significantly correlated with

any other morphological trait, and q-value correction removed significance from all correlations involving head dimensions and caecum mass.

Discussion

The four main goals of this study were to (1) determine the limits of aerobic metabolism and sprint speed in Mongolian gerbils, and test for interactions among these limits; (2) ascertain the extent to which voluntary locomotor behavior is constrained by physiological performance limits; (3) compare energy costs of transport for voluntary versus forced locomotion; and (4) test for associations between complex whole-animal performance traits and the sizes of 'subordinate' effectors (visceral organs, brain, and the musculoskeletal system). To put our results into an appropriate context, it is useful to compare the locomotor and aerobic physiology of gerbils with that of other small mammals.

Basal metabolic rates of our gerbils (1.05 ml O₂/min for a 67.7 g animal) were considerably lower than a previous measurement for *M. unguiculatus* (2.4 ml O₂/min for the same mass; Wang et al., 2000), and somewhat less than predicted by several allometries for BMR in rodents. Back-transformation from log-log allometric regressions can lead to errors (Hayes and Shonkwiler, 2006), so we make comparisons with log₁₀ values; i.e., our value of log₁₀ BMR (ml O₂/min) for a 67.7 g gerbil is 0.0212 and the Wang et al. (2000) value is 0.380. For the same mass and units, Hinds and Rice-Warner (1992) predicted a log₁₀ BMR of 0.164 in non-heteromyid rodents, and Bozinovic (1992) estimated a log₁₀ BMR of 0.152 from an analysis of 29 species, primarily from South America. A recent study of 57 populations from 46 species (Rezende et al., 2004a) predicted a log₁₀ BMR of 0.225 for a gerbil-sized rodent, using a model that included adjustments for phylogenetic relationships. The intraspecific mass exponent of 1.16 for gerbil BMR was higher than the expected interspecific scaling exponent of approximately 0.75, but the 95% confidence interval (.469 – 1.84) includes 0.75.

Our finding that RMR was slightly but significantly lower than BMR is puzzling, as validation tests with steady-state nitrogen dilution indicated high accuracy in $\dot{V}O_2$ measurements. However, it is possible that unexpectedly low RMR values may be artifacts from a combination of poor mixing in the corners of the wheel enclosure's home cage (where gerbils often slept; unpublished data), coupled with position changes and the instantaneous correction applied to gas exchange calculations. It is also possible that despite the lack of a strong circadian activity cycle in captive gerbils (Lerwill, 1974; Sun and Jing, 1984), we would have obtained lower BMR had we measured it during the day instead of at night. However, most of the minimal RMR occurred during the day (25 of 40). The results nevertheless indicate that temperatures in the wheel enclosures (25.1 ± 0.96 °C) were within or close to the thermal neutral zone of gerbils (26-38 °C according to Wang et al., 2000).

In comparison with other small mammals, Mongolian gerbils are intermediate in athletic ability. Exercise $\dot{V}O_{2\max}$ in gerbils (11.2 ml O₂/min for a 67.7 g animal; $\log_{10} = 1.049$) is almost identical to the 11.3 ml O₂/min ($\log_{10} = 1.053$) predicted by a recently compiled allometry for maximum running $\dot{V}O_2$ in a wide size and taxonomic range of mammals (Weibel et al., 2004). Given their low BMR and average $\dot{V}O_{2\max}$, gerbils have a relatively large factorial aerobic scope for exercise (10.7). In comparison, equations for rodent exercise $\dot{V}O_{2\max}$ and BMR from Hinds and Rice-Warner (1992) give an estimated scope of 6.5. If the Weibel et al. (2004) $\dot{V}O_{2\max}$ estimate is substituted, the estimated scope is 7.7 (all of these values are higher than most estimates of thermogenic aerobic scopes: typically 5-6 in warm-acclimated rodents; e.g., Bozinovic, 1992).

Estimated maximal aerobic speeds (MAS) of gerbils during forced exercise (7.82 ± 1.43 km/h, body mass = 67.7 g) are higher than the value of 4.88 km/h predicted from the allometric equation for 39 species of mammals provided by Garland et al. (1988), but within the range of variation for rodents in their sample (e.g., see their Fig. 3). Gerbils were fairly slow sprinters, with maximum sprint speeds averaging 10.8 km/h,

compared to a mean of 13.4 km/h for 14 species of quadrupedal North American rodents (8.9 – 112 g; Djawdan and Garland, 1988; see also Garland et al., 1988). Thus, the MAS of gerbils is a fairly high percentage (75%) of maximum sprint speed. This is roughly comparable to the MAS of 67% of a rather low sprint speed in one strain of laboratory mice (*Mus domesticus*; 3.4 versus 5.1 km/h; Dohm et al., 1994, Girard et al., 2001). However, the MAS of 5.45 km/h in deer mice (*Peromyscus maniculatus*) running at 25 °C is only 41% of their sprint speed of 13.4 km/h (Djawdan and Garland, 1988; Chappell et al., 2004). Across a broad range of mammals, sprint speeds typically average 2-3-fold higher than MAS, and the two measures are generally uncorrelated after controlling for the correlation of each with body mass (Garland et al., 1988).

Aerobic and sprint performance limits: In recent years there has been considerable discussion of functional or evolutionary relations among performance traits, especially the upper and lower limits to aerobic metabolism (a well-known example is the ‘aerobic capacity’ model for the evolution of endothermy; Bennett and Ruben, 1979; Bennett, 1991), and trade-offs between sprint and aerobic performance that might affect evolutionary responses to selection on speed or power output (e.g., Garland et al., 1988; Garland, 1994; Vanhooydonck et al., 2001; Vanhooydonck and Van Damme, 2001; Van Damme et al., 2002; Syme et al., 2005).

Results from a number of studies of the relationship between BMR and $\dot{V}O_{2\max}$ in birds and mammals do not reveal a clear pattern (Table 6). Part of the inconsistency derives from use of dissimilar techniques for eliciting maximum $\dot{V}O_2$: forced exercise and acute cold exposure. The two methods usually do not necessarily yield the same maximum $\dot{V}O_2$ (e.g., Chappell and Bachman, 1995; Rezende et al., 2005), and in small mammals the differences between $\dot{V}O_{2\max}$ in cold and exercise are often enhanced by cold acclimation (Hayes and Chappell, 1986; Chappell and Hammond, 2004; Rezende et al., 2004b). Even if non-uniform methodologies are avoided or accounted for, there are

difficult interpretive issues in analyses of relationships between BMR and $\dot{V}O_{2\max}$ (see Hayes and Garland, 1995 for a review of the 'aerobic capacity' model).

In the present study, perhaps the most salient finding about the sprint and aerobic physiology of gerbils was the paucity of significant correlations among $\dot{V}O_{2\max}$, BMR, RMR, and sprint speed, as well as among other metabolic and locomotor traits (Table 3). BMR was independent of $\dot{V}O_{2\max}$ and RMR, and sprint performance was independent of BMR, RMR, and $\dot{V}O_{2\max}$. The latter finding contrasts with a significant positive correlation between sprint speed and $\dot{V}O_{2\max}$ in a sample of 35 male laboratory mice (Friedman et al., 1992). Factorial scope (a measure of the expandability of aerobic power production; $\dot{V}O_{2\max}/\text{BMR}$), was independent of all other metabolic and locomotor variables except the estimated maximal aerobic speed (MAS). An absence of relationships among these traits might be expected if trait variance was low. However, variance (CV) in gerbil aerobic limits (BMR and $\dot{V}O_{2\max}$; 8.8 and 16.2%; Table 2) was similar to that observed in species with significant correlations among these indices (e.g., deer mice: Hayes, 1989; Belding's ground squirrels, *Spermophilus beldingi*: Chappell and Bachman, 1995; house sparrows, *Passer domesticus*: Chappell et al., 1999). Variation in sprint performance (18.7%) was of similar magnitude. These findings suggest that enhanced sprint speed or increased aerobic exercise capacity in gerbils will not elicit penalties such as burst-versus-endurance performance trade-offs or increased maintenance costs (at least within the limits of trait variation in our study population).

Voluntary locomotor behavior: The Mongolian gerbils in this study ran considerably less than several other rodent species that have been tested in the enclosed-wheel metabolic chambers. The mean time spent running and distance covered by gerbils was 83 min and 1.2 km/day, compared to 126 min and 3 km/day in deer mice Chappell et al., 2004), 319 min and 4.9 km/day in random-bred control (C) lines of laboratory mice, and 373 min and 8.6 km/day in lab mouse lines selected for high voluntary running

distance (S lines; Rezende et al., 2006). Gerbils also ran less than several species of wild-caught rodents (least chipmunks *Tamias minimus*, Panamint kangaroo rats *Dipodomys panamintinus*, golden-mantled ground squirrels *Spermophilus lateralis*, and Belding's ground squirrels, M. A. Chappell, unpublished data), although individual variation was substantial. A possible caveat is that we used only male gerbils in the present study. In some species (lab mice; Swallow et al., 1998; Koteja et al., 1999a,b; Rezende et al., 2006) females run more extensively than males, although this is not always the case (deer mice; Chappell et al. 2004).

Relationships between running speed and metabolic rate (e.g., Taylor et al., 1982) indicate that although high speeds require correspondingly high rates of energy expenditure, they result in the lowest absolute cost of transport (the energy necessary to move a given mass a given distance, independent of speed). Accordingly, the most economical running speed that avoids problems of extensive anaerobic power production should be the maximal aerobic speed (MAS). Free-living golden-mantled ground squirrels (*Spermophilus saturatus*) appear to minimize transport costs by preferentially traveling at speeds close to their MAS (Kenagy and Hoyt, 1988; 1989), but our gerbils did not do this in running wheels. MAS in gerbils is about 8 km/h, while voluntary running speeds in the wheel enclosures (1-minute averages) were strongly biased towards speeds < 1 km/h, rarely reached 3 km/h, and never reached 4 km/h, or 50% of MAS (Figs. 3, 4). Even the highest instantaneous speeds (from 1.5-sec sampling intervals) did not exceed 60% of MAS. Absence of sprinting (speeds > MAS) and extensive use of low and intermediate speeds were also characteristic of voluntary locomotion in deer mice (Chappell et al., 2004), laboratory house mice (Girard et al., 2001; Rezende et al. 2006), and several species of wild rodents (unpublished data).

Most of the distance traveled by gerbils was accomplished at speeds < 2 km/h, and the distributions of voluntary speeds and the distance-vs.-speed relationships in gerbils (Fig. 2) are qualitatively similar to those for deer mice (Chappell et al., 2004). The mean voluntary speed of gerbils (.90 km/h) was intermediate between that of C lines of lab mice (.86 km/h) and both deer mice and S lines of lab mice (1.35 and 1.38 km/h, respectively), even though gerbils are two- to three-fold larger than these mice. Perhaps coincidentally, the voluntary running distance in our study was similar to the average daily movement reported for free-living Mongolian gerbils (1.2 - 1.8 km; Naumov and Lobachev, 1975).

Consistent with the data on voluntary running speeds, voluntary 1-minute maxima for oxygen consumption ($\dot{V}O_{2v1}$) were always well below the aerobic capacity of gerbils, averaging about 42% of $\dot{V}O_{2max}$ (Fig. 1). This is considerably less than corresponding values for two other rodent species tested in the same enclosed wheel respirometer. In deer mice running at 25 °C, $\dot{V}O_{2v1}$ averaged 72% of $\dot{V}O_{2max}$ (Chappell et al., 2004), and in lab mice measured at similar temperatures, $\dot{V}O_{2v1}$ averaged 70% - 80% of $\dot{V}O_{2max}$ (C and S lines, respectively; Rezende et al., 2005). As mentioned above, some of the difference may be attributable to our use of male gerbils, because female laboratory mice run longer and faster than males. Given that gerbils, as well as deer mice and laboratory mice, voluntarily run well within their aerobic limits, the lack of correlation between voluntary running behavior and $\dot{V}O_{2max}$ is not surprising. Generally similar findings were reported for laboratory rats (*Rattus norvegicus*) by Lambert et al. (1996): voluntary running performance in untrained rats could not be predicted by results from treadmill tests of sprint speed or $\dot{V}O_{2max}$, and even after training there was no correlation between voluntary running and $\dot{V}O_{2max}$. However, we found a weak correlation between maximum treadmill-elicited sprint speed and maximum voluntary speed (Table 3), and Friedman et al. (1992) reported consistent (but

not statistically significant) positive correlations between wheel running and $\dot{V}O_2\text{max}$ in male laboratory mice.

Locomotor energetics and cost of transport: The generally linear relationship between running speed and metabolic rate in gerbils undergoing forced exercise, and the elevated intercept of the speed-versus-metabolism regression with respect to resting metabolism (the 'postural cost' of locomotion; Taylor et al., 1970), are qualitatively very similar to results from a broad range of species measured during treadmill locomotion (Taylor et al., 1982). However, gerbils are economical runners: the slope (incremental COT) for gerbils undergoing forced exercise (slope in $\text{kJ kg}^{-1} \text{km}^{-1} = 15.7$) was substantially less than predicted by an allometric equation (slope = 25.1; Taylor et al. 1982, equation 8 transformed to $\text{kJ kg}^{-1} \text{km}^{-1}$ using 20.1 J per ml O_2). The slope for gerbils performing voluntary exercise (slope = 19.5) was somewhat greater than for forced exercise, but still less than the allometric prediction.

Perhaps of greater interest than the lower-than-predicted slopes is the lower zero-speed intercept for voluntary running than for forced running (Fig. 4). Given that BMR was about $18.7 \text{ kJ kg}^{-1} \text{h}^{-1}$ and both forced and voluntary exercise were performed at temperatures within or close to thermoneutrality (Wang et al., 2000), the 'postural cost' for voluntary exercise is about $20 \text{ kJ kg}^{-1} \text{h}^{-1}$ (108% of BMR), compared to about $57 \text{ kJ kg}^{-1} \text{h}^{-1}$ (305% of BMR) for forced exercise. We presume that the $37 \text{ kJ kg}^{-1} \text{h}^{-1}$ difference in postural costs (and the associated divergence in absolute costs of transport, at least at low and moderate speeds) results from higher anxiety, fear or stress during forced exercise than during voluntary exercise. If that conjecture applies universally, then many published data on costs of transport – which are based almost exclusively on forced running protocols – may be elevated above the 'true' (voluntary) running costs experienced by animals under natural conditions. Conceivably the lower slopes during forced exercise may also be an effect of stress, but there are many other factors

that differ between wheel and treadmill running (next paragraphs) that might account for the difference.

Consistent with our results for gerbils, Rezende et al. (2006) found higher zero-speed intercepts for forced than for voluntary exercise in laboratory mice. However, much more data from a variety of species are needed to explore these issues thoroughly, and at least two important caveats apply. First, our gerbils (and the mice used by Rezende et al., 2006) were treadmill-tested without prior training and conditioning. In most treadmill-based studies, animals were trained for extended periods prior to measurements, such that stress during forced exercise might have been ameliorated (e.g., Taylor et al, 1982 trained animals to run on a treadmill for "a period of weeks to months"). For example, Taylor et al. (1982) used data from 62 treadmill-tested species to generate allometric regressions for running costs. Their equation 7 (transformed to $\text{kJ kg}^{-1} \text{h}^{-1}$ using $20.1 \text{ J per ml O}_2$) predicts an intercept of 49.1 for a 67.7 g animal, which is less than our value of 75.8 in untrained gerbils during forced exercise, but more than our value of 38.9 during voluntary exercise.

Second, regardless of the effects of training or stress, comparisons between wheel and treadmill tests are potentially problematic for several reasons, as discussed in detail in Chappell et al. (2004). In brief, (a) treadmill data are usually from steady-state running at constant speeds while voluntary running in species thus far studied is typically intermittent with variable speeds, (b) wheels have momentum that allows 'coasting' (Koteja et al., 1999a) but reduces acceleration, and (c) treadmill running is normally on a level substrate whereas animals in wheels can change between level, uphill, or downhill running. It could be argued that these factors are more likely to influence the slope of the speed-versus-metabolic rate regression than its intercept. However, our results, and similar data for deer mice (Chappell et al., 2004; unpublished data) and for laboratory mice (Rezende et al., 2006) suggest that slopes (COT_{INC}) do not differ substantially between forced and voluntary locomotion.

Only a small fraction of the energy used daily by gerbils was spent on wheel running. On average, incremental running costs (= daily run distance in km * COT_{INC}) were $6.7 \pm 3.8\%$ of average daily metabolic rate (ADMR). Surprisingly, that is almost identical to the fraction of ADMR reported for deer mice that ran more than three times as far per day (6.3%, Chappell et al., 2004), and is similar to fractional locomotor costs in two strains of laboratory mice running 4X and 10X as far as gerbils (4.4% and 7.5% of ADMR, respectively; Koteja et al., 1999b). The similarity in running costs as a percentage of ADMR in mice and gerbils, despite large differences in running distance, is probably due in part to the relatively low metabolic rates of gerbils when not using wheels. Deer mice were frequently active (judged by high and variable $\dot{V}O_2$) during periods when no wheel-running occurred (Chappell et al., 2004); this was uncommon in gerbils (unpublished data). Consequently, ADMR at 25 °C was only 50% higher in gerbils than in deer mice (50.1 and 33.3 kJ/day, respectively) despite a 3-fold difference in body mass; temperatures were within or close to thermoneutrality for both species. Our animals also had considerably lower ADMR than was previously reported for Mongolian gerbils at similar temperatures (89.4 kJ/day in 74.1 g animals housed in large cages but without wheels at 25 °C). At that ADMR, our incremental running costs would be 3.8% of daily energy use. Although low, all of these values are substantially larger than the predicted 'ecological cost of transport' of 0.66% of ADMR for a 67.7 g mammal (Garland, 1983).

Performance and subordinate morphological traits: The behavioral and physiological capabilities of intact animals must reflect characteristics of their organs and tissues, and a number of studies have shown that individual differences in performance are correlated with variation in relative organ size. In endotherms, much of the work has concerned BMR in birds, with particular interest in the role of central (visceral) support organs versus peripheral effectors such as skeletal muscle. Several early papers that

examined intraspecific variation (e.g., Daan et al. 1990; Piersma et al., 1996) suggested that BMR is largely determined by the metabolic output of visceral organs, but subsequent studies of both mammals and birds have revealed little consistency in the specific organs that correlate with BMR (Table 7; for a related study on frogs see Steyermark et al., 2005). In one recent study of deer mice, individual organs were largely uncorrelated to BMR, but the combined mass of visceral organs was positively correlated to BMR while the opposite was true for musculoskeletal mass (Russell and Chappell, 2006). A smaller group of intraspecific tests have explored morphological correlations with the other extreme of aerobic performance, maximum $\dot{V}O_2$ (Table 7; for studies of lizards, snakes, and frogs see John-Alder, 1983; Garland, 1984; Garland and Else, 1987; Garland and Bennett, 1990). Again, few consistencies are apparent, other than an unsurprising positive relationship between muscle mass and exercise $\dot{V}O_{2max}$ in two bird species. Few similar data are available for other performance traits in mammals, such as sprint speed and jumping ability (Table 7).

Statistically significant correlations between whole-animal performance and physiology, organ masses, and head, foot, and body linear dimensions were absent in our male gerbils (Table 4, Fig. 5), despite considerable variance in both performance and morphology. We were particularly surprised to find no relation between $\dot{V}O_{2max}$ and either the peripheral effector organs primarily responsible for high rates of oxygen consumption (the musculoskeletal system, which comprised 40-54% of total body mass) or the central visceral organs most directly involved in oxygen uptake and delivery (heart and lungs).

It is difficult to draw strong conclusions from an absence of correlative associations between structure and function. Our results do not implicate the masses of specific central or peripheral organs, or even pooled visceral or musculoskeletal organs, as controlling factors for aerobic or sprinting performance. It is likely that traits we did not measure (hematocrit, enzyme function, total limb dimensions, capillary geometry,

mitochondrial density, muscle fiber type, etc.) play crucial roles in setting performance limits. It is also conceivable that our protocols for testing performance did not push animal to their limits. There was no evidence for this, however, and the repeatability of sprinting tests, as well as considerable experience with the techniques used to measure sprint speed and $\dot{V}O_{2\max}$ convince us that our data are robust.

Relationships among organ sizes: Several of the correlations among body-mass corrected organ sizes (Table 5) are intuitively consistent with integrated functions. For example, many organs responsible for food, nutrient, and waste processing were positively correlated (liver, stomach, intestine, kidney). Also, the size of the musculoskeletal system, which is responsible for most aerobic power production, was positively correlated with size of visceral organs responsible for oxygen delivery, nutrient processing, and waste processing (heart, liver, and kidney, respectively). Testis size correlated positively with several organs, but not with the size of other reproductive structures or the musculoskeletal system. Interestingly, there were numerous significant positive correlations among organ size, but only one significant negative correlation (large intestine versus other reproductive tissues). That suggests gerbils generally do not 'trade-off' mass allocations among visceral organs, or between visceral organs and the musculoskeletal system. For example, a hypothetical conditioning regime favoring increased proportional musculoskeletal mass would not necessarily be expected to adversely affect digestive organs or reproductive structures, at least in terms of organ size.

A recent report found considerably plasticity in the size of visceral organs (particularly digestive organs) in Mongolian gerbils in response to changes in diet quality (Liu and Wang, 2007). Compared to gerbils fed high-quality diets, animals maintained for 14 days on a low-quality (high fiber) diet did not differ in body mass or digestible energy intake, but the length and wet mass of the gut was significantly larger.

The authors did not measure muscle or musculoskeletal mass, but they ascribed reduced carcass mass in animals on low-quality diets to loss of adipose tissue, rather than to decreases in skeletal muscle mass.

Conclusions: We found no indication that aerobic capacity constrains voluntary locomotor behavior in Mongolian gerbils, similar to results from two other small mammals (Chappell et al., 2004; Rezende et al., 2006). Our data also do not support the hypothesis that animals should preferentially run at near-maximal aerobic speeds in order to minimize costs of transport (of course, food was available *ad libitum* in these studies). Mechanistically, and of potential importance for evolution, we found no evidence of trade-offs between capacities for sprinting and aerobic power production, or of increased maintenance costs (BMR) in individuals with higher sprint or aerobic performance. Thus, increased sprint speed or aerobic capacity would not be expected to affect BMR. One possible reason for the lack of a trade-off between sprinting and stamina-type locomotion is that gerbils are rather average in terms of both types of performance, whereas trade-offs may be restricted to extreme performers (see Garland, 1994, pp. 268-270).

The generally linear relation between speed and metabolic rate in gerbils, and the elevated zero-speed intercept relative to resting metabolism ('postural cost') resembled that of other terrestrial runners. However, we found a difference in postural cost, and hence absolute costs of transport at the speeds used by gerbils, between forced and voluntary running: postural cost was higher during forced exercise. Our data revealed no linkages between sprint and aerobic performance limits and the size of either central or peripheral organs.

Our results are consistent with many other studies that have found weak correlations between organismal performance (e.g., $\dot{V}O_{2\max}$) and putatively relevant subordinate traits. They also bolster the conclusions of Garland and Huey (1987, p.

1407), who, in a critique of symmorphosis, wrote that "Some components within a system may exhibit 'excessive construction' (Gans, 1979), whereas others constitute limiting factors. Furthermore, given the vicissitudes of evolutionary change, factors that are limiting (or in excess) may well differ among species (or populations)."

Acknowledgments: We are grateful to Leslie Karpinski, Scott A. Kelly, and Dr. Laura McGeehan for gerbil care. The work was supported by U.C. Riverside academic senate research awards, NSF IOB-0543429 (T. Garland, Jr.), and NSF IBN-0111604 (K. A. Hammond and M. A. Chappell). W. Saltzman was supported in part by NIH grant MH60728. We thank E. Hice and J. Urrutia in the UCR Biology machine shop for constructing metabolism chambers and wheel enclosures. The comments of two anonymous reviewers helped us improve the original version of the paper.

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Table 1. Descriptive statistics of physiological, behavioral, and morphological variables in Mongolian gerbils. Abbreviations: BMR: basal metabolic rate; RMR: resting metabolic rate; $\dot{V}O_{2\max}$: maximum oxygen consumption; RER: respiratory exchange ratio at $\dot{V}O_{2\max}$; $\dot{V}O_{2v1}$: maximum 1-min average oxygen consumption during voluntary running; $V_{\max1}$: maximum 1-min average speed during voluntary running; ADMR: average daily metabolic rate; D_{run} : distance traveled in 24 h; T_{run} : time spent running in 24 h; SRL: snout-rump length; foot: mean hind foot length; headW: head width; headL: head length; SI: small intestine; LI: large intestine; repro: other reproductive tissues (spermatic cord, prostate, etc.); GN: gastronemius muscle complex; motor: musculoskeletal system (less head, tail, and feet).

| Variable | Units | Range | Mean, SD | N |
|--------------------|------------------------|--------------|---------------|----|
| body mass | g (live mass) | 52.3 – 78.9 | 67.7 ± 6.0 | 40 |
| BMR | ml O ₂ /min | .75 – 1.45 | 1.07 ± .21 | 27 |
| RMR | ml O ₂ /min | .70 – 1.22 | .935 ± .116 | 40 |
| $\dot{V}O_{2\max}$ | ml O ₂ /min | 9.14 – 15.3 | 11.3 ± 1.30 | 40 |
| RER | (ratio) | .69 – 1.31 | .97 ± .12 | 38 |
| spont speed | km/h | 6.97 – 14.5 | 10.8 ± 1.98 | 34 |
| $\dot{V}O_{2v1}$ | ml O ₂ /min | 3.9 – 7.10 | 5.11 ± .73 | 40 |
| $V_{\max1}$ | km/h | 1.53 – 3.86 | 2.20 ± .47 | 40 |
| ADMR | ml O ₂ /min | 1.35 – 2.13 | 1.73 ± .16 | 40 |
| distance | km/day | 285 – 4953 | 1237 ± 887 | 40 |
| time | min/day | 21.7 – 206.9 | 88.3 ± 38.7 | 40 |
| SRL | mm | 104 – 137 | 118 ± 8.0 | 40 |
| foot | mm | 22.0 – 29.2 | 27.0 ± 1.3 | 40 |
| headL | mm | 35.0 – 42.0 | 38.1 ± 1.4 | 40 |
| headW | mm | 18.0 – 22.0 | 19.9 ± 0.8 | 40 |
| heart | g (wet mass) | .180 – .313 | .237 ± .037 | 40 |
| lungs | g (wet mass) | .488 – .907 | .674 ± .097 | 40 |
| liver | g (wet mass) | 2.34 – 4.02 | 3.20 ± .42 | 40 |
| kidney | g (wet mass) | .521 – .743 | .625 ± .064 | 40 |
| spleen | g (wet mass) | .0393 – .139 | .0608 ± .0166 | 40 |
| stomach | g (wet mass) | .327 – .571 | .451 ± .057 | 40 |
| SI | g (wet mass) | .362 – .760 | .514 ± .087 | 40 |
| caecum | g (wet mass) | .225 – .591 | .328 ± .077 | 40 |
| LI | g (wet mass) | .162 – .472 | .331 ± .075 | 40 |
| testes | g (wet mass) | .74 – 1.18 | 1.02 ± .10 | 40 |
| repro | g (wet mass) | .50 – 1.75 | 1.26 ± .27 | 40 |
| brain | g (wet mass) | .82 – 1.03 | .943 ± .052 | 40 |
| GN | g (wet mass) | .82 – 1.47 | 1.17 ± .16 | 37 |
| motor | g (wet mass) | 24.4 – 38.3 | 31.6 ± 3.20 | 40 |

Table 2. Allometry of measured traits with body mass and age, from regressions of \log_{10} -transformed variables. Values for CV are the SD of residuals in regressions of \log_e -transformed data, which approximates CV (see text). Abbreviations: vCOTint and vCOTslp = intercept and slope of voluntary cost of transport; tCOTint and tCOTslp = intercept and slope of treadmill COT; other abbreviations, units, and sample sizes as in Table 1. P values < .05 are **boldfaced**.

| Variable | Constant ± SE | Mass coefficient ± SE | Mass P | Age coefficient ± SE | Age P | R ² | F, P | CV, % |
|---------------------|------------------|--------------------------|------------------|-------------------------|---------------|----------------|------------------------|-------|
| BMR | -4.98 ± 1.50 | 1.22 ± .36 | .0027 | -0.00069 ± .00153 | .65 | .319 | 5.63, .010 | 16.2 |
| RMR | -.770 ± .386 | .458 ± .217 | .042 | -0.000815 ± .00038 | .037 | .158 | 3.47, .042 | 11.2 |
| $\dot{V}O_2$ max | .107 ± .697 | .631 ± .171 | .00071 | -0.00279 ± .00068 | .00021 | .386 | 11.6, .00012 | 8.8 |
| $\dot{V}O_2$ sprint | 3.08 ± 1.63 | -.164 ± .394 | .68 | -0.00026 ± .00159 | .87 | .008 | .12, .89 | 18.7 |
| $\dot{V}O_2$ v1 | -.147 ± 1.05 | .398 ± .258 | .13 | .00076 ± .00103 | .47 | .099 | 2.03, .15 | 13.2 |
| RER | .707 ± .843 | .064 ± .47 | .89 | .0014 ± .0007 | .068 | .100 | 1.94, .16 | 8.2 |
| Vmax1 | -.65 ± 1.37 | .982 ± .336 | .0059 | .00083 ± .0013 | .54 | .232 | 5.57, .00765 | 17.3 |
| vCOTint | 1.66 ± .31 | -.118 ± .171 | .49 | .072 ± .083 | .39 | .03 | .48, .62 | 3.8 |
| vCOTslp | 1.50 ± .70 | -.278 ± .389 | .48 | .146 ± .188 | .44 | .02 | .43, .66 | 8.5 |
| tCOTint | 3.50 ± .98 | -.624 ± .521 | .24 | -.238 ± .272 | .39 | .08 | 1.46, .25 | 11.6 |
| tCOTslp | -2.93 ± 1.39 | 1.63 ± .74 | .034 | .541 ± .385 | .17 | .21 | 4.49, .019 | 16.6 |
| ADMR | -2.19 ± .56 | .679 ± .138 | <.0001 | -0.00104 ± .00055 | .065 | .399 | 12.3, <.0001 | 7.1 |
| D _{run} | -.45 ± 4.37 | 1.93 ± 1.07 | .079 | -0.00032 ± .0043 | .94 | .09 | 1.77, .19 | 54.9 |
| T _{run} | -.75 ± 3.88 | 1.42 ± .95 | .14 | -0.00179 ± .0038 | .64 | .06 | 1.11, .34 | 48.8 |
| SRL | 1.18 ± .17 | .131 ± .042 | .00363 | .00713 ± .0216 | .0022 | .456 | 15.1, <.0001 | 4.9 |
| foot | 2.02 ± .33 | .307 ± .080 | .00515 | -0.00016 ± .00032 | .61 | .30 | 7.57, .0018 | 4.1 |
| headL | 2.80 ± .27 | .145 ± .064 | .030 | .0479 ± .033 | .16 | .217 | 5.13, .011 | 3.3 |
| headW | 2.39 ± .30 | .223 ± .070 | .00297 | -.0700 ± .037 | .065 | .237 | 5.58, .0077 | 3.6 |
| heart | -2.76 ± .39 | 1.142 ± .217 | <.0001 | .0185 ± .112 | .17 | .457 | 15.6, <.0001 | 11.5 |
| lungs | -2.20 ± .38 | 1.099 ± .208 | <.0001 | .00445 ± .107 | .97 | .456 | 15.5, <.0001 | 4.6 |
| liver | -1.12 ± .37 | .818 ± .205 | .00030 | .061 ± .105 | .56 | .347 | 9.8, .00038 | 4.6 |
| kidney | -1.55 ± .29 | .575 ± .159 | .00089 | .140 ± .082 | .094 | .374 | 11.0, .0002 | 3.54 |
| spleen | -2.04 ± .77 | .204 ± .426 | .64 | .209 ± .219 | .35 | .041 | .79, .46 | 9.5 |
| stomach | -1.50 ± .39 | .293 ± .216 | .18 | .295 ± .111 | .011 | .251 | 6.20, .0047 | 4.8 |
| SI | -1.61 ± .52 | .315 ± .289 | .28 | .352 ± .149 | .023 | .202 | 4.7, .016 | 6.5 |
| caecum | -2.62 ± .67 | 1.36 ± .37 | .00074 | -.177 ± .19 | .36 | .269 | 6.8, .0031 | 8.3 |
| LI | -1.70 ± .76 | .009 ± .422 | .98 | .571 ± .217 | .012 | .172 | 3.86, .030 | 9.4 |
| testes | -1.30 ± .27 | .670 ± .151 | <.0001 | .0405 ± .078 | .61 | .391 | 11.9, <.0001 | 3.4 |
| repro | -2.75 ± .73 | 1.207 ± .403 | .0048 | .302 ± .207 | .153 | .293 | 7.68, .0016 | 9.0 |
| brain | -.568 ± .166 | .348 ± .092 | .00054 | -.0452 ± .047 | .344 | .280 | 7.20, .0023 | 2.1 |
| GN | -1.46 ± .49 | .910 ± .266 | .00166 | -.0672 ± .125 | .595 | .257 | 5.89, .0063 | 5.3 |
| motor | -.119 ± .218 | .909 ± .121 | <.0001 | -.0217 ± .062 | .728 | .690 | 30.6, <.0001 | 2.8 |

Table 3. Pairwise Pearson correlations (see Table 1 for sample sizes) among performance variables (based on residuals from regressions on body mass and age). Abbreviations: vCOT int and vCOT slope: intercept and slope, respectively, for costs of transport in voluntary running; tCOT int and tCOT slope: same but for forced treadmill running; vMAS: estimated maximum aerobic speed in voluntary locomotion; tMAS: same but for forced treadmill running; other abbreviations as in Table 1. In each cell, the top value is r and the bottom value is P; correlations with nominal $P < .05$ are **boldfaced**. After Bonferroni adjustment, P values $< .0005$ remain significant. The positive FDR statistic $\pi_0 = .726$ indicates that P values $\leq .0062$ remain significant (indicated by asterisks*).

| | RMR | $\dot{V}O_{2max}$ | scope | $\dot{V}O_{2v1}$ | ADMR | sprint | vCOT int | vCOT slp | tCOT int | tCOT slp | vMAS | tMAS | Vmax1 | D _{run} | T _{run} |
|-------------------|--------------|-------------------|-----------------------------|------------------------------|-------------------------------|---------------|------------------------------|--------------------------------|-----------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------|----------------------------------|----------------------------------|
| BMR | .047 .817 | -.101 .615 | -.762 <.0001* | .023 .908 | -.099 .623 | -.128 .533 | .223 .263 | -.299 .130 | .083 .699 | .042 .845 | .177 .377 | -.128 .552 | -.026 .899 | .086 .671 | .052 .796 |
| RMR | | -.170 .293 | -.143 .478 | -.312 .0498 | .565 .00015* | .216 .219 | .196 .238 | -.042 .804 | -.246 .148 | .027 .874 | -.128 .451 | -.167 .361 | -.29 .069 | -.09 .58 | -.094 .562 |
| $\dot{V}O_{2max}$ | | | .496 .0086 | -.109 .502 | .136 .403 | -.002 .991 | .058 .728 | -.021 .901 | .014 .935 | -.066 .709 | .501 .0013* | .501 .0025* | .119 .465 | .180 .265 | .182 .260 |
| scope | | | | -.217 .276 | .056 .781 | .138 .503 | .005 .980 | .163 .417 | -.001 .997 | -.279 .187 | .109 .587 | .503 .012 | .004 .984 | -.050 .806 | .033 .872 |
| $\dot{V}O_{2v1}$ | | | | | -.101 .534 | -.094 .599 | -.125 .453 | .160 .339 | -.304 .080 | .173 .327 | -.236 .154 | -.202 .251 | .307 .054 | .004 .980 | -.039 .811 |
| ADMR | | | | | | .146 .412 | .436 .0062* | -.170 .309 | -.376 .028 | .146 .411 | .172 .301 | .069 .698 | .074 .650 | .383 .015 | .361 .022 |
| sprint | | | | | | | .381 .031 | -.100 .587 | .300 .101 | .092 .624 | .095 .606 | -.179 .334 | -.353 .041 | -.183 .300 | -.214 .224 |
| vCOT int | | | | | | | | -.543 .00043* | -.064 .727 | -.070 .704 | .444 .0052* | .136 .457 | -.041 .805 | .269 .102 | .045 .789 |
| vCOT slope | | | | | | | | | -.096 .603 | .107 .558 | -.864 <.0001* | -.084 .649 | -.131 .431 | -.320 .050 | -.041 .805 |
| tCOT int | | | | | | | | | | -.643 <.0001* | .129 .481 | .346 .045 | -.418 .014 | -.208 .237 | -.166 .348 |
| tCOT slope | | | | | | | | | | | -.135 .462 | -.851 <.0001* | .144 .418 | .106 .552 | -.012 .948 |
| vMAS | | | | | | | | | | | | .289 .109 | .088 .601 | .259 .117 | .038 .819 |
| tMAS | | | | | | | | | | | | | .042 .813 | .084 .639 | .180 .307 |
| Vmax1 | | | | | | | | | | | | | | .628 <.0001* | .403 .0098 |
| D _{run} | | | | | | | | | | | | | | | .798 <.0001* |

Table 4. Correlations (see Table 1 for sample sizes) between performance and morphology (based on body mass + age residuals). Dry organ masses were used here; results were qualitatively similar for wet organ masses. Abbreviations as in Tables 1 and 2. In each cell, the top value is r and the bottom value is P; raw correlations with $P < .05$ are **boldfaced**. Both the Bonferroni adjusted α (.0002) and the positive FDR statistic ($\pi_0 = .704$; significant $P < .0002$) indicate that none of the P values in the table are significant.

| | BMR | RMR | VO ₂ max | VO ₂ v1 | ADMR | sprint | vCOT int | vCOT slp | tCOT int | tCOT slp | vMAS | tMAS | Vmax1 | D _{run} | T _{run} |
|---------|-----------------------------|----------------------------|-----------------------------|------------------------------|---------------|-----------------------------|---------------|---------------|----------------------------|-----------------------------|----------------------------|-----------------------------|---------------|------------------|------------------|
| SRL | -.171 .404 | -.021 .897 | -.006 .969 | -.284 .080 | -.024 .887 | .119 .511 | .116 .493 | .262 .117 | -.034 .848 | .375 .026 | -.215 .208 | -.311 .083 | -.160 .330 | -.043 .795 | .169 .305 |
| foot | .018 .930 | -.078 .637 | -.123 .457 | .076 .644 | -.093 .572 | -.198 .269 | .023 .893 | -.033 .898 | -.043 .807 | .120 .494 | -.012 .945 | -.153 .404 | .087 .599 | -.020 .982 | -.108 .515 |
| headL | .178 .374 | .033 .841 | .053 .746 | .207 .201 | .282 .078 | -.180 .309 | -.014 .933 | -.100 .550 | .003 .988 | -.293 .083 | .107 .530 | -.124 .499 | .144 .377 | -.102 .531 | -.054 .740 |
| headW | .137 .505 | -.164 .317 | -.131 .427 | .150 .363 | -.204 .214 | .148 .412 | -.309 .062 | -.327 .066 | .355 .036 | -.145 .407 | .363 .030 | .062 .740 | -.011 .948 | -.213 .193 | -.218 .182 |
| heart | -.141 .482 | .038 .817 | .080 .624 | -.216 .181 | -.143 .378 | -.232 .187 | -.048 .774 | -.108 .519 | .285 .092 | -.074 .667 | .138 .414 | .148 .418 | -.068 .677 | -.094 .563 | -.012 .943 |
| lung | -.189 .346 | -.126 .438 | -.041 .800 | .306 .055 | -.030 .854 | .483 .0038 | -.024 .885 | .102 .544 | -.032 .852 | .245 .150 | -.047 .781 | -.120 .513 | .106 .514 | .044 .787 | -.082 .615 |
| liver | .113 .575 | -.047 .774 | .047 .744 | -.160 .325 | -.106 .513 | -.089 .616 | -.178 .285 | -.055 .742 | .145 .398 | -.053 .758 | .037 .828 | -.080 .662 | -.069 .671 | -.036 .825 | .140 .389 |
| kidney | -.170 .397 | -.032 .844 | .145 .371 | -.233 .148 | -.130 .425 | .040 .821 | -.061 .716 | .037 .827 | .109 .258 | .204 .232 | .040 .815 | -.107 .559 | .091 .577 | .036 .827 | .171 .292 |
| spleen | -.171 .395 | .119 .463 | -.216 .181 | -.080 .624 | -.187 .249 | .046 .796 | -.213 .200 | .218 .189 | .182 .288 | -.038 .824 | -.296 .075 | -.017 .926 | -.160 .323 | -.066 .686 | .034 .836 |
| stomach | -.300 .129 | .297 .063 | .120 .460 | -.467 .0024 | .144 .377 | .100 .575 | .193 .245 | .098 .559 | .024 .889 | .004 .984 | -.091 .593 | -.175 .338 | -.257 .109 | -.077 .636 | .074 .651 |
| SI | .119 .553 | .088 .588 | .291 .069 | -.360 .023 | -.052 .751 | -.133 .454 | .006 .970 | -.137 .412 | .317 .060 | -.238 .161 | .300 .071 | .228 .210 | -.121 .456 | -.037 .819 | .023 .888 |
| caecum | -.028 .889 | .338 .033 | -.329 .038 | -.190 .241 | .108 .505 | -.027 .880 | .116 .488 | -.145 .386 | .023 .869 | -.079 .645 | -.025 .883 | -.227 .212 | -.245 .128 | -.040 .809 | -.131 .421 |
| LI | .011 .955 | .079 .628 | .215 .180 | -.121 .455 | .143 .378 | .075 .673 | .027 .871 | .145 .384 | .137 .424 | .146 .397 | -.147 .386 | -.093 .613 | -.038 .816 | .161 .322 | .249 .122 |
| testes | -.391 .044 | -.013 .939 | -.130 .425 | -.063 .698 | -.022 .892 | .161 .363 | -.183 .272 | .061 .718 | .025 .883 | .016 .927 | -.191 .259 | -.040 .829 | .097 .551 | .194 .231 | .266 .097 |
| repro | .150 .454 | -.294 .066 | .009 .957 | .204 .206 | -.137 .400 | -.119 .503 | -.109 .515 | -.251 .129 | .329 .050 | -.371 .026 | .281 .093 | .313 .081 | .126 .440 | .006 .970 | -.016 .920 |
| brain | -.029 .885 | -.130 .422 | -.023 .887 | .348 .028 | -.060 .716 | .025 .889 | -.272 .098 | .250 .131 | -.177 .301 | .345 .039 | -.207 .218 | -.386 .029 | .044 .788 | -.120 .461 | -.181 .264 |
| GN | .317 .131 | -.075 .661 | .105 .538 | .208 .217 | -.168 .320 | .169 .362 | -.208 .231 | .156 .371 | .307 .082 | -.191 .288 | .014 .936 | .063 .746 | -.227 .177 | -.193 .254 | -.040 .815 |
| motor | .137 .496 | -.240 .136 | .156 .336 | -.049 .765 | -.053 .746 | .015 .933 | .046 .782 | -.207 .212 | .365 .029 | -.067 .700 | .280 .093 | -.028 .878 | -.132 .415 | -.129 .429 | .127 .433 |

Table 6. Survey of correlations between BMR and $\dot{V}O_2\text{max}$ in birds and mammals, after effects of body mass were removed. N is the number of species in interspecific comparisons or the number of individuals in intraspecific comparisons. Significant correlations (2-tailed $P < .05$) are indicated by asterisks (*).

| Taxa | Species, N | BMR – $\dot{V}O_2\text{max}$ correlation | $\dot{V}O_2\text{max}$ elicited with: | Reference |
|---------|--|--|---------------------------------------|--|
| Birds | Interspecific, passerines N = 10 | 0.86* | Cold exposure | Dutenhoffer & Swanson, 1996 |
| | Interspecific N = 24 | 0.87* | Cold exposure | Rezende et al. 2002 |
| | Interspecific, tropical species N = 19 N = 31 | 0.132 0.387* | Cold exposure Forced exercise | Wiersma, P, Chappell, M. A., Williams, J. B.; unpublished data |
| | House sparrow, <i>Passer domesticus</i> N =30 | 0.44* | Forced exercise | Chappell et al., 1999 |
| | Red junglefowl, <i>Gallus gallus</i> males N= 36 females, N = 36 | -0.330 -.046 | Forced exercise | Hammond et al., 2000 |
| | Red knot, <i>Calidris canutus</i> N=26 | r not stated; P > 0.5 | Cold exposure | Vézina <i>et al.</i> , 2006 |
| Mammals | Interspecific, mammals N = 18 | -0.14 | Cold exposure | Koteja, 1987 |
| | Interspecific, rodents N = 8 | 0.59 | Cold exposure | Koteja, 1987 |
| | Interspecific, shrews N=13 | 0.25 | Cold exposure | Sparti, 1992 |
| | Interspecific, rodents N = 29 | 0.46* | Cold exposure | Bozinovic, 1992 |
| | Interspecific, rodents N = 9 | 0.70* | Cold exposure | Hinds & Rice-Warner, 1992 |
| | Interspecific, rodents N=17 | 0.64* | Forced exercise | Hinds & Rice-Warner, 1992 |
| | Interspecific, rodents N = 46 species, 57 populations | 0.534* | Cold exposure | Rezende et al., 2004a |
| | Belding's ground squirrel, <i>Spermophilus beldingi</i> N = 95 | 0.31* 0.005 | Forced exercise Cold exposure | Chappell & Bachman, 1995 |
| | Lab mouse, <i>Mus domesticus</i> N = 60 | -.069 | Forced exercise | Hayes et al., 1992 |
| | Deer mouse, <i>Peromyscus maniculatus</i> N =50 | 0.318* | Cold exposure | Hayes, 1989 |

Table 7. Survey of correlations between whole-animal aerobic and locomotor traits and subordinate morphological traits in birds and mammals, after effects of body mass were removed statistically. Plus-signs (+) indicate significant ($P \leq 0.05$) positive correlations; minus-signs (-) indicate significant negative correlations, and 0 indicates no significant correlation. Note that the various studies did not all measure the same organs.

| Taxa | Species | Trait | Suborganismal correlates | Reference |
|---------|---|------------------------|---|-----------------------------|
| Birds | Interspecific | BMR | Heart, kidney + | Daan et al., 1990 |
| | Red knot, <i>Calidris canutus</i> | BMR | Lean body mass + | Piersma et al., 1996 |
| | great knot, <i>Calidris tenuirostris</i> | BMR | Flight muscle, intestine + | Battley et al., 2001 |
| | Tree swallow, <i>Tachycineta bicolor</i> | BMR | Kidney + Intestine - | Burness et al., 1998 |
| | House sparrow, <i>Passer domesticus</i> | BMR | Kidney, liver, gonad + leg and pectoralis muscle + Intestine, heart 0 | Chappell et al., 1999 |
| | Red junglefowl, <i>Gallus gallus</i> | BMR | Spleen + (females) Lung, lung, stomach, caecum + (males) | Hammond et al., 2000 |
| | House sparrow | $\dot{V}O_2\text{max}$ | leg and pectoralis muscle + (adults) heart + (juveniles) other visceral organs 0 | Chappell et al., 1999 |
| | Red junglefowl | $\dot{V}O_2\text{max}$ | Heart, leg and pectoralis muscle +; caecum - other visceral organs 0 (males) large intestine, hematocrit + other visceral organs, muscle 0 (females) | Hammond et al., 2000 |
| mammals | Laboratory mouse, <i>Mus domesticus</i> | RMR | Kidney, liver, heart, intestine + | Konarzewsky & Diamond, 1995 |
| | Laboratory mouse | RMR | Digestive organs + | Speakman & McQueenie, 1996 |
| | Field vole, <i>Microtus agrestis</i> | BMR | Heart + | Meerlo et al., 1997 |
| | Deer mouse, <i>Peromyscus maniculatus</i> | BMR | No organ mass correlates | Koteja, 1996 |
| | Deer mouse | BMR | Combined visceral organs + Musculoskeletal system - | Russell & Chappell, 2007 |
| | Wild mouse, <i>Mus musculus</i> , laboratory mouse, crosses | $\dot{V}O_2\text{max}$ | Heart + Liver, gastrocnemius, hematocrit 0 | Dohm et al., 1994 |
| | Laboratory mouse X wild mouse | Sprint speed | Gastrocnemius mass 0 Muscle fiber type 0 | Garland et al., 1995 |
| | Domestic cat, <i>Felis domesticus</i> | jumping | Limb length + Muscle mass 0 | Harris & Steudel, 2002 |

Figure legends:

Fig. 1. Aerobic performance in relation to body mass in Mongolian gerbils. The upper and lower limits to oxygen consumption are shown (basal metabolic rate, BMR over 10 min, and maximal oxygen consumption during forced exercise, $\dot{V}O_{2\max}$ over 1 min, respectively), along with two measures of aerobic performance during voluntary activity: average daily metabolic rate (ADMR, the 24-hour average of oxygen consumption) and the highest 1-min average of voluntarily-attained $\dot{V}O_2$ ($\dot{V}O_{2m1}$).

Fig. 2. Distributions of voluntary running behavior in 40 Mongolian gerbils, each measured over ~23.5 h. Data are 1-minute averages separated by 3-minute gaps (to avoid autocorrelation; see text). A: frequency distribution time spent running at various speeds. B: distance traveled at various speeds. The bin size is 0.1 km/h for both.

Fig. 3. Effect of averaging interval on maximal values of running speed (A) and oxygen consumption (B) in 40 Mongolian gerbils during voluntary wheel running. Dashed lines indicate regressions of 2- and 5-minute averages (unfilled and filled circles, respectively) against 1-minute averages (regressions were forced through the origin). Slopes are fractions of 1-minute values; for example, a slope of .834 indicates 83.4% of the 1-minute value. The solid line shows a slope of 1.0 (equivalent X and Y values).

Fig. 4. Forced and voluntary running energetics in Mongolian gerbils (pooled data from 40 individuals). The open circles and dashed regression line show values from forced treadmill exercise, with each point representing one individual at one speed. The solid

circles and solid regression line show values from voluntary exercise. Regression coefficients and r^2 values are means from individuals (not from pooled data). Points from voluntary exercise are 1-min averages separated by 3-min intervals to avoid autocorrelation (see text). For comparison, the maximum power output in forced exercise is about $196 \text{ kJ Kg}^{-1} \text{ h}^{-1}$.

Fig. 5. Distributions of P values in relation to the π_0 statistic (the overall proportion of true null hypotheses: Storey and Tibshirani 2003; Storey 2003) for the correlations in Tables 3-5. The bin size is .05; π_0 is shown as dashed lines. A random set of P-values would produce a flat histogram with all bins showing a relative frequency close to 1.0; the presence of non-null hypotheses (i.e., significant tests after accounting for Type 1 errors) is indicated by high relative frequencies in the left-most bin. Values of π_0 were computed with the R program 'QVALUE'.

Figure 1:

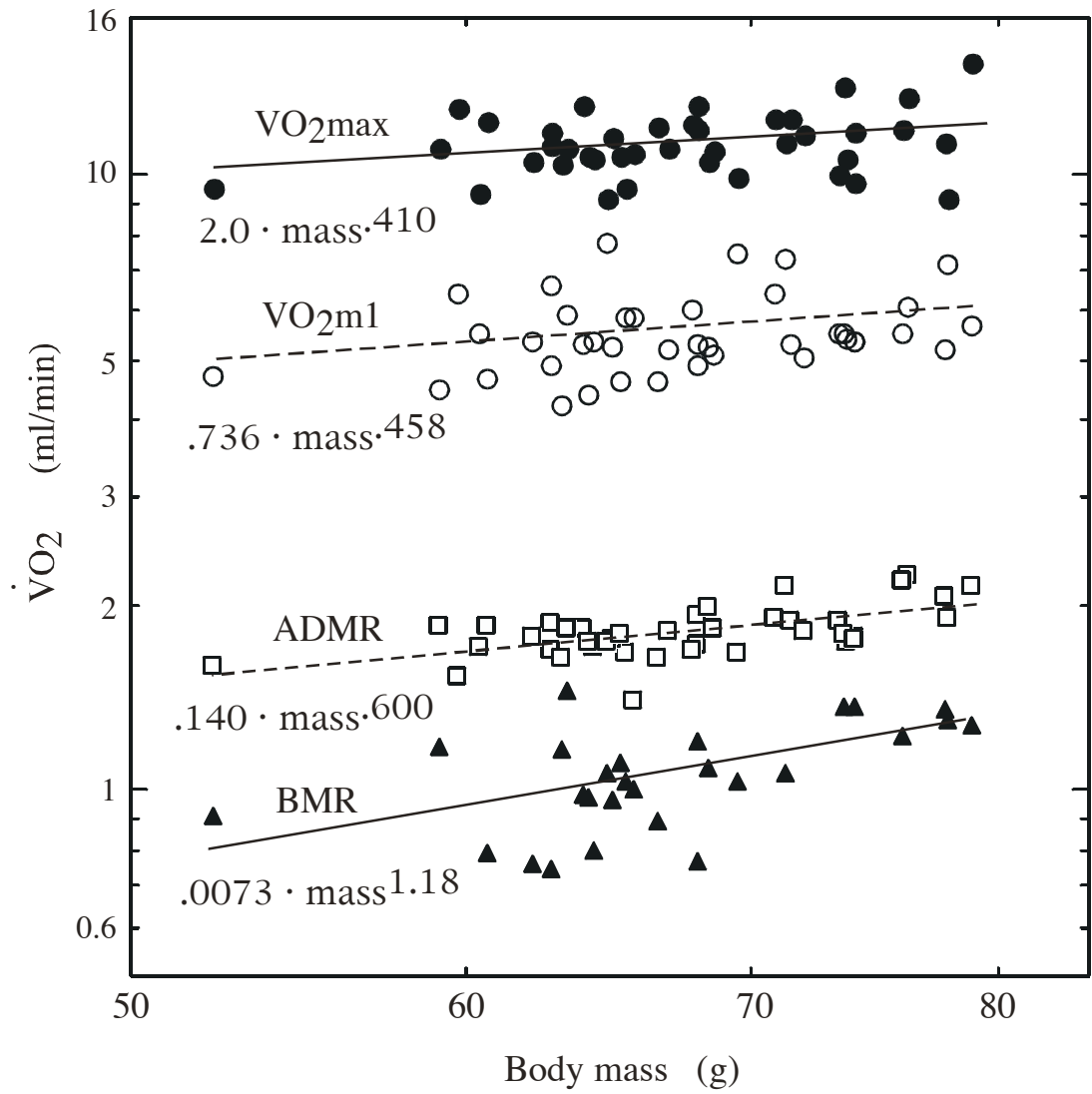


Figure 2:

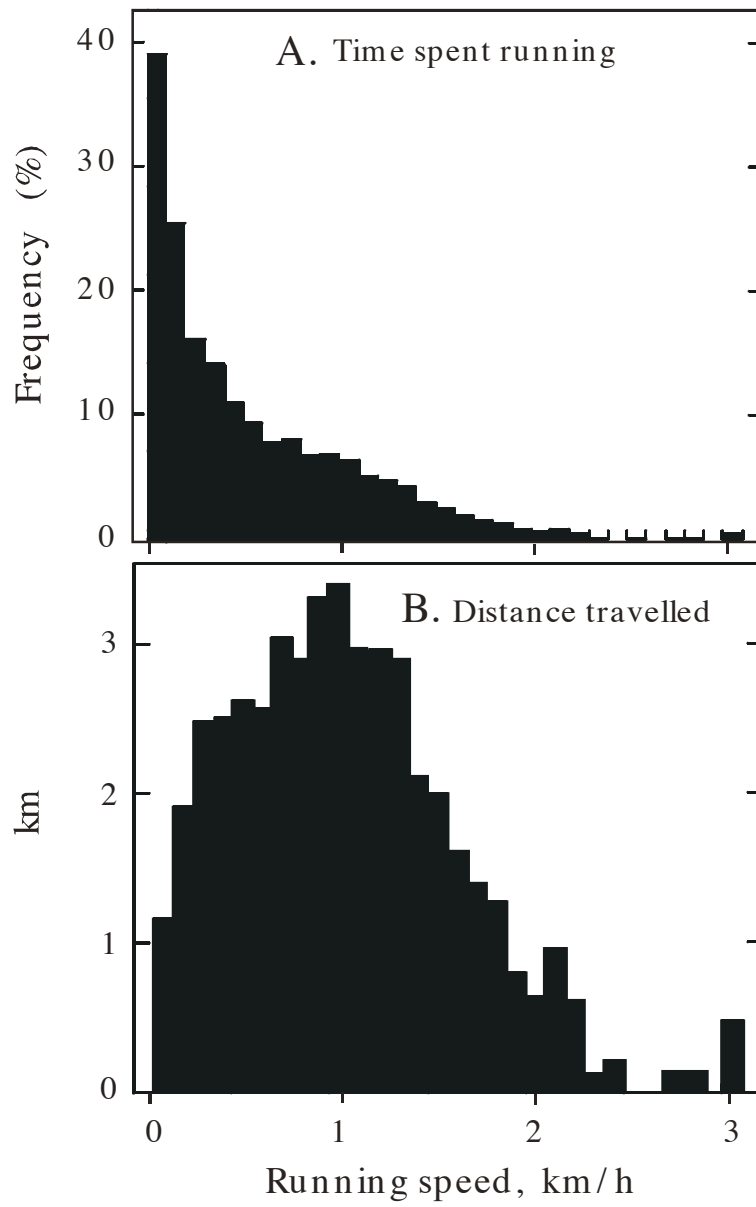


Figure 3:

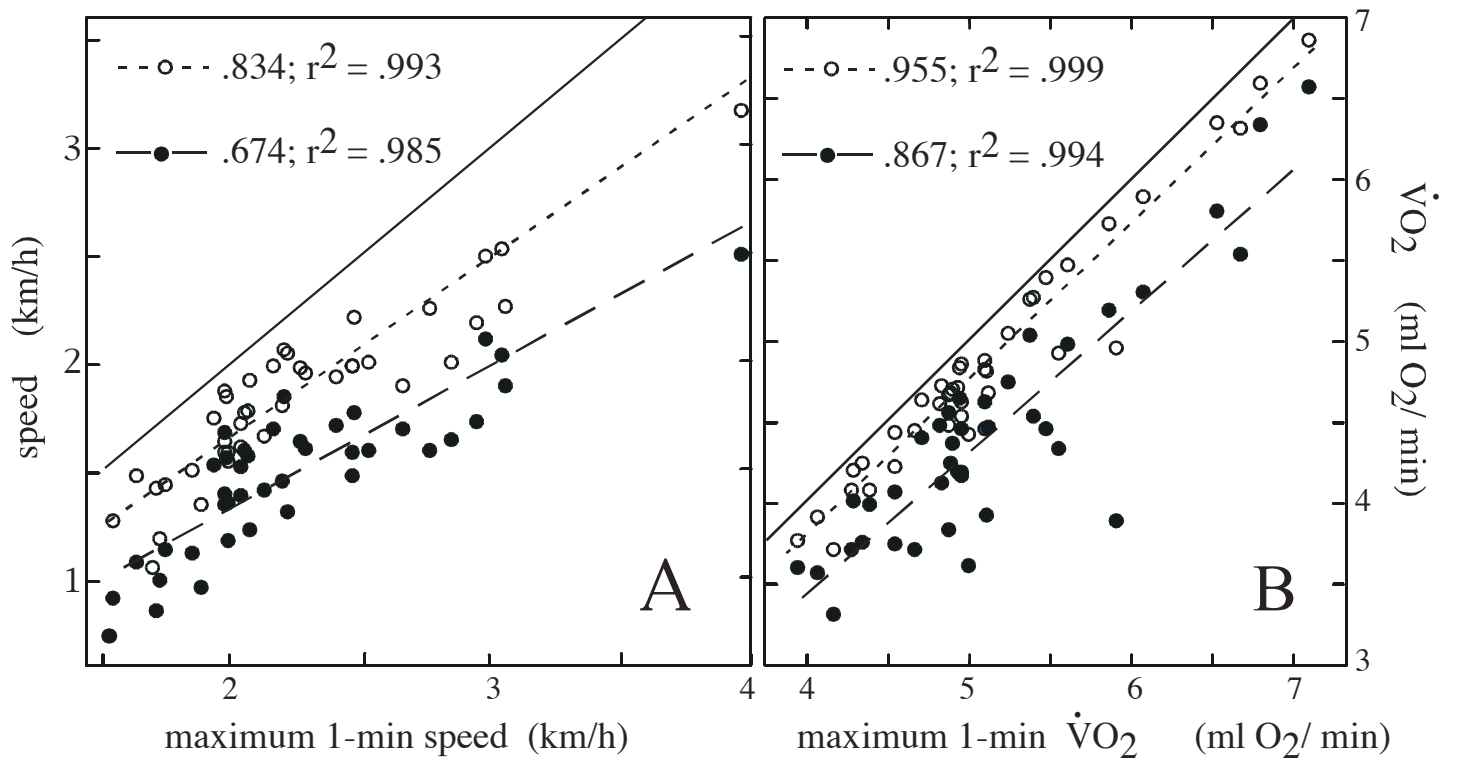


Figure 4:

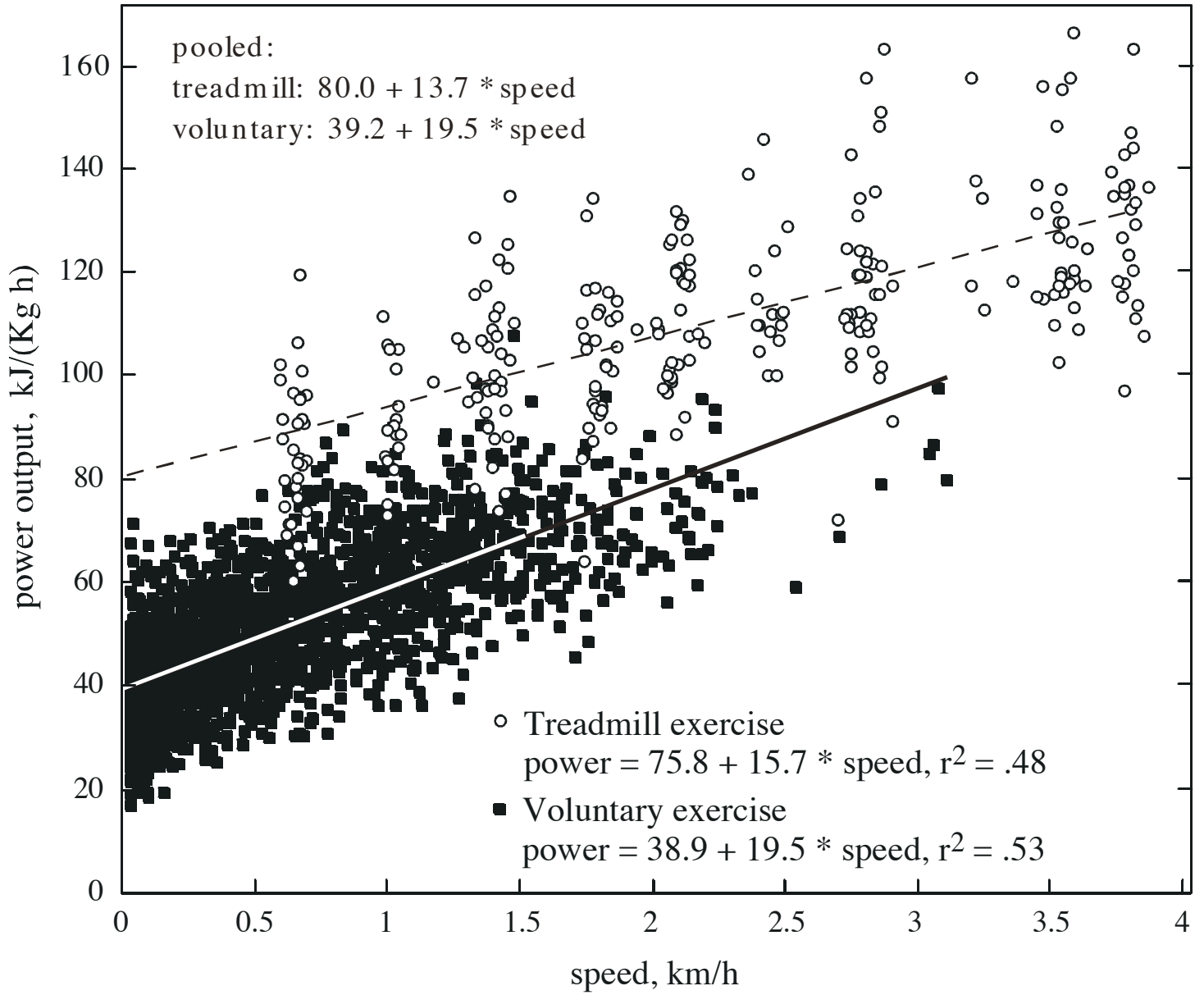


Figure 5:

