Selective breeding for high voluntary exercise in mice increases maximal (VO_2max), but not basal metabolic rate

Nicole E. Schwartz[1], Monica P. McNamara[1], Jocelyn M. Orozco[1], Jaanam O. Rashid[1], Angie P. Thai[1], Theodore Garland, Jr.[1]

[1]Department of Evolution, Ecology, and Organismal Biology, University of California, Riverside, Riverside, CA, USA

Author for Correspondence:
Theodore Garland, Jr.
Department of Evolution, Ecology, and Organismal Biology
University of California, Riverside, Riverside, CA, 92506 USA
Phone: 951-827-3524
tgarland@ucr.edu

Abstract

In general, sustained high rates of physical activity require a high maximal aerobic capacity (VO_2max), which may also necessitate a high basal aerobic metabolism (BMR), given that the two metabolic states are linked via shared organ systems, cellular properties, and metabolic pathways. We tested the hypotheses that (a) selective breeding for high voluntary exercise in mice would elevate both VO_2max and BMR, and (b) these increases are accompanied by increases in the sizes of some internal organs (ventricle, triceps surae muscle, liver, kidney, spleen, lung, brain). We measured 72 females from generations 88 and 96 of an ongoing artificial selection experiment comprising 4 replicate High Runner (HR) lines bred for voluntary daily wheel-running distance and 4 non-selected Control (C) lines. With body mass as a covariate, HR lines as a group had significantly higher VO_2max (+13.6%, $P < 0.0001$), consistent with previous studies, but BMR did not significantly differ between HR and C lines (+6.5%, $P = 0.181$). Additionally, HR mice did not statistically differ from C mice for whole-body lean or fat mass, or for the mass of any organ collected (with body mass as a covariate). Finally, mass-independent VO_2max and BMR were uncorrelated ($r = 0.073$, $P = 0.552$) and the only statistically significant correlation with an organ mass was for VO_2max and ventricle mass ($r = 0.285$, $P = 0.015$). Overall, our results indicate that selection for a behavioral trait can yield large changes in behavior without proportional modifications to underlying morphological or physiological traits.

KEYWORDS: Basal Metabolic Rate, Behavior, Endothermy, Energetics, Experimental Evolution, Locomotion

Introduction

Metabolic rate, i.e., the rate at which organisms acquire and expend energy, is a fundamental aspect of an animal's physiology, forming a link from the first principles of physics and chemistry to the biology of individual organisms (Brown et al. 2004; Lovegrove 2019). For vertebrates, two key boundaries of animal energetics are the maximal rate of oxygen consumption attained during exercise (VO_2max) and the resting (RMR) or basal (BMR) metabolic rate (usually also measured as O_2 consumption) (Hulbert and Else 2004). In general, these extremes set the upper and lower bounds for energy expenditure (although exceptions exist, e.g., cold-induced summit metabolism in small mammals can exceed VO_2max (Chappell and Hammond 2004; Andrew et al. 2019)), and some (e.g., Biro et al. 2018) have argued that the difference between these bounds (i.e., aerobic scope) may constrain variability in the expression of some behaviors. A recent meta-analysis found that various measures of whole-animal metabolic rate (including resting and maximal metabolic rates) were not strongly related to aspects of movement behavior (e.g., activity in familiar environments, exploration of novel environments, dispersal) at the level of individual variation (Wu and Seebacher 2022). From a macroevolutionary perspective, Boratyński (2020) compared 52 species of mammals and found that home range size (corrected for body size) was positively correlated with VO_2max but negatively correlated with BMR, thus suggesting that "aerobic scope plays a prominent role in constraining home ranges" (p. 468) (see also Albuquerque et al. 2015). A potential link between metabolic rate and behavior underlies the aerobic capacity model for the evolution of vertebrate endothermy, which posits that directional selection favored high levels of sustained aerobic physical activity, which required an increase in VO_2max and, due to unspecified linkages with VO_2max, also increased BMR (Bennett and Ruben 1979; Taigen 1983; Hayes and Garland 1995).

On first principles, maximal and resting rates of O_2 consumption should be positively correlated, given that the two metabolic states share many organ systems (e.g., cardiovascular), cellular properties (e.g., mitochondrial density), and metabolic pathways (Bennett and Ruben 1979). First principles do not, however, support a 1:1 correlation between VO_2max and BMR (as pointed out by Taigen (1983)), given that different tissues account for the bulk of O_2 consumption at rest versus during activity. During sustained activity, skeletal muscle is responsible for the bulk of O_2 consumption, but has a relatively low metabolic rate when an animal is at rest (Weibel et al. 2004). At rest, O_2 is mainly consumed by visceral organs and the brain (Konarzewski and Diamond 1995; Książek et al. 2004; Konarzewski and Książek 2013), whose collective metabolic

rates are relatively low during sustained, aerobically supported activity. Nevertheless, the brain and some visceral organs (e.g., heart, liver) may remain quite active during periods of activity and have been consistently correlated with VO_2max and/or BMR at the level of individual variation (e.g., Garland 1984; Konarzewski and Diamond 1995; Chappell et al. 1999; Książek et al. 2004; Rezende et al. 2006b; Gębczyński and Konarzewski 2009; Konarzewski and Książek 2013), which should result in some degree of positive correlation between the two metabolic states.

Alternatively, a mechanistic link between VO_2max and BMR may stem from cellular properties. Mitochondria consume O_2 and produce ATP via a series of protein complexes embedded in its inner membrane. This inner membrane can be "leaky," decoupling O_2 consumption and ATP production. This leakiness is a major contributor of BMR (Else and Hulbert 1987; Else et al. 2004). Therefore, higher mitochondrial densities could provide the capacity for higher rates of O_2 consumption, but at the cost of a higher resting rate of O_2 consumption (Else and Hulbert 1981; Hulbert and Else 1989; Hulbert et al. 2006), although the general application of this as a unifying explanation is debated (Konarzewski and Książek 2013).

Empirical studies have tested for a positive correlation between VO_2max and BMR at several levels. For example, an allometric comparison indicated that both VO_2max and BMR average ~6 times higher in mammals as compared with lizards, with the ratio of VO_2max/BMR or SMR being ~9 for both lineages (Garland and Albuquerque 2017, see their Table 1). Among species within lineages, a comprehensive comparative analysis of 176 vertebrate species (including fish, amphibians, reptiles, birds, mammals) found a positive correlation between residual VO_2max and BMR or SMR (Nespolo et al. 2017). Among individuals within species, Pough and Andrews (1984) found no correlation between residual exercise VO_2 and either standard or resting rates of O_2 consumption in the lizard *Chalcides ocellatus* (see also: Garland 1984), whereas Chappell and Bachman (1995) reported a significant positive correlation between residual VO_2max and BMR in the wild rodent *Spermophilus beldingi*. With respect to quantitative genetics, Dohm et al. (2001) reported a positive additive genetic covariance between residual VO_2max and BMR in an outbred strain of laboratory house mice, but only in a reduced model containing additive and environmental variance. Hence, the authors advocated that their results be interpreted with a degree of caution, given potential biases resulting from imposed modelling constraints. Similarly, Sadowksa et al., (2005) found that the additive genetic covariance between VO_2max and BMR in bank voles was also positive, and was significant across several models (i.e., potentially more robust). Overall, interspecies comparisons generally report a positive correlation

between maximal and resting rates of O_2 consumption, whereas comparisons within species are less consistent (Auer et al. 2017; Nespolo et al. 2017).

One fairly direct way to test for correlations among physical activity behavior, whole-animal metabolic rates, and lower-level traits is through replicated selection experiments, which allow for the study of evolution in real time and in response to well-defined and reproducible selective regimes (Swallow and Garland 2005; Swallow et al. 2009a,b; Storz et al. 2015). Several selection experiments have tested for a positive relationship between VO_2max, BMR, and lower-level traits. For example, Książek et al. (2004) selectively bred two lines of laboratory mice for high versus low mass-corrected BMR. Starting at generation 7, the between-line difference in mass-corrected BMR increased, and after 19 generations of divergent selection, the between-line difference in BMR was 8.9 mLO_2/h, equivalent to ~2.3 phenotypic standard deviations. This increase in BMR (+18%) was accompanied by a larger small intestine, liver, kidneys, and heart in the high-BMR mice. However, the low-BMR mice had significantly higher (+4%) VO_2max (elicited by forced swimming) than those from the high BMR group, contradicting the idea that VO_2max and BMR are positively related (Książek et al. 2004). Similarly, Gębczyński and Konarzewski (2009) found that 10 generations of selection for high body-mass corrected VO_2max (elicited by forced swimming) in laboratory mice resulted in a 12% increase in VO_2max, but no change in BMR. Additionally, VO_2max was positively correlated with higher masses of gastrocnemius muscles and heart, but not other visceral organs (intestine, stomach, liver, and kidneys). Using a colony of wild-derived bank voles (*Myodes glareolus*), Sadowksa et al. (2015) conducted a multiway artificial selection experiment meant to mimic an adaptive radiation, with four lines each bred for either high aerobic metabolism during forced swimming, predatory behavior on crickets, or ability to maintain body mass on a low-quality plant diet. After 11 generations, mass-corrected VO_2max and BMR were both significantly higher in the four swimming-selected lines as compared with four non-selected control lines, although the magnitude of these increases differed greatly (+49% in VO_2max, +7.3% in BMR). Finally, Wone et al. (2015) bred four replicate lines of laboratory house mice for high mass-independent VO_2max during forced treadmill exercise, four other lines for high VO_2max and low BMR, and maintained four non-selected controls. After eight generations, VO_2max significantly increased (+11%) in lines bred for high VO_2max, while BMR had not significantly changed (+2.5%). In the antagonistically selected lines, VO_2max increased (+5.3%) while BMR decreased (-4.2%, not statistically significant), which, while it does not falsify the notion that VO_2max and BMR may be linked, provides support for the independent evolution of the metabolic traits.

None of the aforementioned selection experiments directly tested the specific scenario proposed by the aerobic capacity model (Bennett and Ruben 1979), which has been more broadly interpreted as suggesting a fundamental link between physical activity behavior, VO_2max, and BMR that is of general applicability to vertebrates (Taigen 1983; Hayes and Garland 1995). We address this scenario with a well-established mouse model in which four replicate High Runner (HR) lines have been bred for high voluntary wheel-running behavior and are compared with four non-selected Control (C) lines (Swallow et al. 1998a; Garland 2003; Wallace and Garland 2016). In a sample of females from generations 88 and 96, we measured maximal and basal rates of O_2 consumption, and recorded the mass of the kidneys, spleen, liver, brain, heart (ventricles), and lungs. We hypothesized that: (1) selection for high voluntary exercise would have resulted in an increased VO_2max for HR mice, (2) that HR mice would also have an increased BMR, and (3) that some organs (e.g., brain, heart, liver), which are quite active during aerobic exercise as well as under basal conditions, would be increased in HR mice. Although several previous studies have reported elevated VO_2max in the HR lines (e.g., Rezende et al. 2005, 2006b,a; Kolb et al. 2010; Dlugosz et al. 2013b), only one previous study reported BMR (of aged individuals), finding no statistical difference between the HR and C lines (Kane et al. 2008).

Materials and methods

(a) Mouse model

For logistical reasons, we sampled from two generations of an ongoing selection experiment for high voluntary wheel-running behavior (Swallow et al. 1998a; Garland 2003; Careau et al. 2013; Wallace and Garland 2016): 50 females from generation 88 and 22 from generation 96. Only females were used because: 1) the number of mice per day that could be tested for VO_2max and BMR was limited, such that a smaller sample size or lengthier testing period were required; and 2) females can be housed four per cage as adults, whereas males often need to be individually housed to prevent fighting. The delay between generations was approximately 2 years and was primarily the result of COVID-19-related restrictions on personnel and research.

Individuals were measured for VO_2max, BMR, and organ masses, but were not exposed to wheels at any time; thus, they represent "baseline" or untrained conditions. Additionally, we collected wheel-running data from siblings that were part of the routine selective breeding procedures. For each generation of the selection experiment, mice are housed four per cage by sex from weaning (21 days of age) until ~6-8 weeks of age, when they are housed individually with access to an activity wheel (1.12-m circumference). Over six days, wheel revolutions are recorded in 1-minute intervals. For the four replicate HR lines, the highest-running male and female from each family are chosen as breeders for the next generation, with no sibling pairings allowed. For the four replicate C lines, breeders are chosen without regard to wheel running (Swallow et al. 1998a; Careau et al. 2013). Animals were maintained in accordance with NIH guidelines, and all procedures were approved by the IACUC of UCR, which is accredited by AAALAC.

The original base population of mice used to start the selection experiment included individuals with hindlimb muscles that were ~50% smaller than normal-muscle individuals (Garland et al. 2002; Houle-Leroy et al. 2003). This “mini-muscle” phenotype is caused by a single nucleotide polymorphism that acts as a Mendelian recessive and was present at a frequency of ~7% in the base population (Kelly et al. 2013). The phenotype was only ever observed in one C line and in two HR lines. The phenotype eventually disappeared from the C line, became fixed in HR line 3, and remains polymorphic in HR line 6 (Hiramatsu et al. 2017; Cadney et al. 2021; Castro et al. 2021). Of the 72 mice used here, all 14 in HR line 3 had the mini-muscle phenotype (as expected) and 5 of the 22 mice in HR line 6 had the mini-muscle phenotype.

Although the mice used within this study never had access to a running wheel, we did have the wheel-running data from their siblings, which were part of the overall selection experiment.

Briefly, mice are housed with access to an exercise wheel (1.12-m circumference) for six days. During this period, we record the wheel revolutions in each 1-minute interval over a period of 23 hours. We then compute the total number of revolutions (i.e., daily running distance), the number of 1-minute intervals that had at least one revolution (i.e., minutes of wheel activity), the mean revolutions per minute (i.e., average running speed), and the maximum revolutions per minute (i.e., maximum running speed) (Koteja and Garland 2001; Hiramatsu and Garland 2018). A measure of wheel freeness is also included as a covariate in analyses of wheel running (e.g., Girard et al. 2007; Kolb et al. 2010).

(b) Maximal O_2 consumption

VO_2max was measured in an enclosed wheel metabolic chamber (effective volume 900 mL; ~15 cm diameter), as described in (Dlugosz et al. 2013a). Briefly, an upstream mass flow controller set incurrent air flow to ~2,000 mL/min. Excurrent air was subsampled at ~150mL/min, scrubbed of H_2O and CO_2 by Drierite and soda lime, respectively, and directed to an O_2 sensor. Data from an O_2 analyzer (Applied Electrochemistry Inc., S-3A) were recorded in 1-second intervals on a computer equipped with a National Instruments A-D converter and LabHelper software (M.A. Chappell, Warthog Systems, www.warthog.ucr.edu).

Each mouse was tested twice, with a day of rest between trials. The repeatability of VO_2 achieved in forced running trials was determined by performing a paired Student's t-test using the raw VO_2 values, which tests for differences in the average values from one day to the next. In addition, after regressing the VO_2 of each trial on its corresponding body mass and age, we performed a paired Student's t-test of the residuals. Pearson's correlation and the associated t- and p-values for these tests are reported below. Mice averaged 58 days of age (range 50-66 days) at the start of testing, were randomized with regard to time of day and testing order, and all tests were performed at 22-25°C during the photophase. Each test was less than 10 minutes and consisted of: 1) 1-minute reference reading of incurrent air at the start; 2) ~1-minute adjustment period after the mouse was placed in the chamber; 3) testing period wherein (a) the wheel was manually propelled by one of two researchers across all measurements, (b) the initial speed was used to elicit a walking pace from the mouse, (c) the researcher accelerated the wheel (by hand) approximately every 30 seconds, (d) which continued until VO_2 did not increase for ~3 minutes or the mouse could not continue running, 4) ~1-minute recovery period before removal from chamber; and 5) 1-minute reference reading. The same protocol was applied to all mice in this study, and so any measurement error should be comparable across individuals. Values reported

here are similar to those previously reported for these mice, both when using a treadmill (Rezende et al. 2005 p. e.g.,; Kolb et al. 2010) or the wheel apparatus (e.g., Claghorn et al. 2017; Cadney et al. 2022).

After every trial, each mouse was given an objective score of exhaustion, indicated by the number of seconds after the trial before the mouse began walking again. These data were analyzed on a scale of 1 to 5, where an exhaustion of 1 indicated that 1 second had elapsed and an exhaustion of 5 indicated that 5 or more seconds had elapsed. Additionally, each mouse was given a subjective score of overall cooperativity, indicated by whether the mouse consistently ran with the direction of wheel rotation, or sometimes ran in the opposite direction. This was also on a scale of 1 to 5, where a cooperativity score of 1 indicated the mouse did not run when prompted, and a cooperativity score of 5 indicated the mouse attempted to run even at the highest speeds. In cases of uncooperative mice (e.g., cooperativity scores of 1 or 2) or technical difficulties, a third trial was conducted (N = 16) and used to replace the poor trial.

Warthog LabAnalyst software recorded $\%O_2$ and flow rate of incurrent air. LabAnalyst was used to smooth metabolic data via a nearest-neighbor algorithm, and the 'instantaneous' transformation was used to resolve rapid changes in respiration (Bartholomew et al. 1981). VO_2 was calculated as:

$$VO_2 = V \times (FIO_2 - FEO_2) / (1 - FEO_2),$$

where V is flow rate (mL/min STP; standard temperature and pressure), and FIO_2 and FEO_2 are the fractional O_2 concentrations in the incurrent and excurrent air, respectively. For each mouse, the highest 1-minute continuous average for each trial was calculated and the highest VO_2 of any trial was used as VO_2max for subsequent analyses.

(c) Basal O_2 consumption

Basal metabolic rate (BMR) was determined by measuring O_2 consumption at rest in postabsorptive mice at ~32°C (within their thermal neutral zone: Lacy and Lynch 1979). The setup for recording BMR was similar to that for VO_2max, except incurrent air flow was ~500mL/min, mice were in plastic respiration chambers (10cm x 7.5cm x 7.5cm), and excurrent air was subsampled at ~100mL/min.

Mice were separated into two groups, and tests began at either 1200- or 1600-hours PST. Mice averaged 68 days of age (range 57-80 days) at the start of testing. Food was removed four hours prior to testing, which is adequate for obtaining a postabsorptive state in mice (Jensen et al. 2013). Mice were tested over a period of 4 hours, wherein excurrent air was subsampled for 45

minutes, then incurrent air was subsampled for 15 minutes. Two mice were measured at a time using separate channels (Sable Systems International., Oxzilla). Four mice were tested each day, over a period of 13 days. Analysis of BMR was the same as for VO_2max, except data were recorded in 2-second intervals and the lowest 5-minute continuous average was used. For each of the lowest values, we verified that the trace was stable, thus indicating that animals were at rest.

(d) Dissection

Mice were euthanized by decapitation without anesthesia (average age 76 days, range = 70-81) and blood samples were immediately collected from the trunk via heparinized micro-hematocrit tubes, then spun in a micro-hematocrit centrifuge for 5 minutes. Approximately four samples were collected for each mouse and readings were averaged. The whole brain, heart ventricles, kidneys, liver, lungs, spleen, and the triceps surae muscle group were each collected and weighed.

(e) Whole-body, lean, and fat mass

All mice were weighed at weaning, before VO_2max, before and after BMR trials (average used), and before dissection. Body composition was measured by non-invasive quantitative magnetic resonance (EchoMRI-100; Echo Medical Systems LLC, Houston, Texas, USA), which independently calculated fat and lean mass, after the first VO_2max trial, after the BMR trial, and before dissection.

(f) Statistical analyses

Statistical analyses were performed using SAS Proc Mixed v15 (SAS Institute, Cary, NC, USA). HR and C lines were compared by a mixed model, using the restricted maximum likelihood (REML) method, with linetype and mini-muscle status as fixed effects. Replicate line (4 HR and 4 C) was nested within linetype as a random effect using the containment method for d.f., such that the d.f. for linetype were always 1 and 6. We tested the significance of the random effect of the replicate lines using the COVTEST option. This yields the estimates, standard errors, and statistical significance of any covariance parameters, which were restricted to non-negative covariance estimates. The effect of replicate line was never statistically significant for any measured trait (Tables 1, 2). For wheel-running traits, separate variances were allowed for HR and C lines, as previous studies have established a greater variability in wheel-running behavior among HR lines (Garland et al. 2011). We also checked for any interactions between body mass and linetype for all measured traits (i.e., heterogeneity of slopes). None were statistically significant, and thus were not included in the final model (i.e., slopes for body mass were assumed to be homogenous). Generation was included as a random effect in preliminary analyses, but was

never statistically significant, and thus was removed from final analyses. Additionally, some values were removed due to known problems (e.g., loss of tissue during dissection, equipment malfunction) prior to analysis. Outliers were removed when the standardized residual was greater than ~3 standard deviations and/or the difference from the next value was greater than ~1 standard deviation. Least Squares Means (LSMs) and associated standard errors are presented to compare HR with C lines and mini-muscle versus normal mice.

Correlations between VO_2max, BMR, and relevant organ masses were calculated in two ways: (1) for individual mice, using the standardized residuals for each trait, derived from each of the SAS Proc MIXED analyses with linetype and mini-muscle status as fixed effects, and line nested within linetype as a random effect; (2) for line means, using the Least Squares Means (LSMs) derived from SAS Proc MIXED analyses with "line" (N = 9, separating mini- and normal-muscle mice in Line HR6) as a fixed effect. Finally, a multiple regression analysis was performed (listwise deletion of data; p to enter = 0.05) to test for combined predictors of VO_2max.

Results

(a) Sibling wheel-running behavior

To avoid any training effect on the comparison of VO_2max, BMR, or organ mass, focal mice did not receive wheel access. However, for their female siblings, HR mice ran ~3-fold more revolutions/day than C mice (Figure 1A, Table S1). This increase in wheel-running behavior was caused primarily by a significant increase in average running speed (+142%: Figure 1C, Table S1), accompanied by a non-significant increase in running duration (+27%: Figure 1B, Table S1). Additionally, maximum running speed was significantly higher in HR mice (+94%: Figure 1D, Table S1).

(b) Body, lean, and fat mass

HR mice did not significantly differ from C mice for whole-body, lean, or lean-adjusted fat mass when measured at VO_2max, BMR, or dissection, although HR mice did have consistently less lean-adjusted fat mass at each measurement (Tables 1 and 2). Mini-muscle mice were significantly smaller when measured at VO_2max, BMR, and dissection, due to decreased lean mass, but also had increased lean-adjusted fat mass at VO_2max, BMR, and dissection (Tables 1 and 2).

(c) Maximal and basal rates of O_2 consumption

The VO_2 achieved in forced running trials was repeatable ($r = 0.821$, $P < 0.0001$), although the latter of the VO_2 trials was consistently higher ($t = 2.554$, $P = 0.013$). Additionally, after regressing each value on its respective body mass, residual VO_2 was also repeatable ($r = 0.751$, $P < 0.0001$).

The mass corrected VO_2max (higher of the two VO_2 values) of HR mice was ~13.6% higher than that of C mice (Figure 2A, Table 1). However, mass-corrected BMR did not significantly differ between HR and C mice, although HR mice had +6.5% higher mass-corrected BMR than C mice (Figure 2C, Table 1). Mini-muscle status did not significantly affect either VO_2max or BMR (Figure 2A and C, Table 1). However, when lean mass was used as a covariate (see above), VO_2max was ~4.6% higher in mini-muscle mice (Figure 2B, Table 1). Mini-muscle mice were also significantly more exhausted after VO_2max (Table 1).

(d) Organ masses

HR mice did not significantly differ from C mice for any mass-adjusted organ mass, nor did they differ in hematocrit; however, they tended to have smaller lungs than C mice (Figure S1, Table 2).

Mini-muscle mice had ~50% less hindlimb muscle mass, as expected (Garland et al. 2002; Houle-Leroy et al. 2003), had significantly larger livers and lungs, and tended to have larger kidneys and spleens (Figure S1, Table 2).

(e) Correlations

VO_2max and BMR were not significantly correlated at the level of residual (individual) variation or for line means (with HR line 6 split into normal and mini-muscle individuals, i.e., based on nine values) (Table 3). However, residual VO_2max and ventricle mass were significantly positively correlated among all individuals and for line means (Table 3). BMR and ventricle mass were significantly positively correlated among line means, but not among individuals (Table 3). Mass-corrected VO_2max and BMR were not significantly correlated with any other lower-level trait (Table 3). In a forward regression analysis (listwise deletion of data; p to enter = 0.05), only residual ventricle mass entered (N = 65). Correlations among organ masses are presented in Supplemental Table S2.

Discussion

Several alternative, though not necessarily mutually exclusive, hypotheses have been proposed to explain the often-observed positive relationship between VO_2max and BMR, at the level of proximate and/or ultimate causation. The aerobic capacity model (Bennett and Ruben 1979) suggests that, with respect to ultimate causation, selection for high levels of sustained aerobic activity would require an increase in VO_2max, and that an increase in BMR would also occur due to hypothetical mechanistic linkages (proximate causation). Although originally proposed in the context of the evolution of avian and mammalian endothermy, this model might also apply more generally. As outlined in the Introduction, empirical studies have provided mixed support for this model at several levels. However, a direct test of the primary assertions of the aerobic capacity model has not previously been conducted.

Here, we used an ongoing artificial selection experiment wherein mice are bred for high voluntary exercise behavior during days 5 and 6 of a 6-day exposure to wheels (Swallow et al. 1998a; Garland 2003; Wallace and Garland 2016). After 10 generations of selection, mice from the HR lines ran, on average, ~75% more revolutions per day than those from the C lines, and had ~7% higher body mass-corrected VO_2max (Swallow et al. 1998a,b), though BMR was not measured. In later generations, HR mice reached a selection limit at which they ran approximately three-fold more than C mice on a daily basis (Careau et al. 2013), which has remained true across tens of generations (e.g., see Singleton and Garland 2019; Cadney et al. 2021; McNamara et al. 2022; present study). HR mice also have higher activity in home cages when housed individually without wheels (Malisch et al. 2009), and higher food consumption, both with and without wheels (Copes et al. 2015; see also Rezende et al. 2009), as compared with C mice. Several additional studies have reported VO_2max, and most have verified higher values for HR lines (Rezende et al. 2005, 2006a,b; Kolb et al. 2010; Dlugosz et al. 2013b). However, the only study of BMR found no significant effect of selection, did not measure VO_2max or any organ masses, and used mice that were far older (~22.5 months) than the normative wheel-testing age (~2 months) for the selection experiment (Kane et al. 2008). Thus, more information has been needed to determine whether the HR mouse model supports the aerobic capacity model of vertebrate energetics. In the present study, HR mice had significantly higher VO_2max (+13.6%, Table 1, Figure 2A), but did not have significantly higher BMR (+6.5%, Table 1, Figure 2C). Additionally, VO_2max and BMR were not correlated at any level (e.g., among individuals or replicate lines, Table 3). Finally, aside from the positive correlation between ventricle mass and VO_2max among individuals (consistent

with a previous study: (Rezende et al. 2006b)) (but not among replicate lines; Table 3), and between ventricle mass and BMR among replicate lines (but not among individuals; Table 3), VO_2max and BMR were not correlated with any other organs. Thus, the two metabolic states do not appear mechanistically linked through the lower-level traits measured here. Overall, our results offer limited support for the aerobic capacity model, consistent with the three rodent selection experiments that targeted VO_2max and/or BMR (see Introduction).

Beyond the aerobic capacity model, our results, and the HR selection experiment as a whole, may offer insights into other hypotheses regarding links between VO_2max, BMR, and other traits (Hayes and Garland 1995; Hillman et al. 2013; Careau et al. 2015; Auer et al. 2017). For example, under the assimilation capacity model (Koteja 2000), selection favors high-intensity parental care, especially the feeding of juveniles, which requires higher daily energy expenditure (e.g., due to foraging (see also Farmer 2000)), and thus an increased rate of energy processing. Here, BMR increases as a correlated response to the increased capacity of the alimentary tract, as these organs are a primary contributor toward BMR (Konarzewski and Diamond 1995; Książek et al. 2004; Konarzewski and Książek 2013). HR mice in the present study did not have statistically larger internal organs (e.g., liver, kidney) (or BMR) and have not been shown to have a larger alimentary tract (Kelly et al. 2017), although mini-muscle mice (a subset of the HR mice) have higher stomach dry mass and longer small intestines (Kelly et al. 2017). Additionally, Koteja (2000) proposed that high daily energy expenditure was driven by high-intensity parental care, which has not been found to differ in an important way between HR and C lines (Girard et al. 2002; Keeney 2011).

In closing, we note that the HR mouse selection experiment is relevant to the "behavior evolves first" model (e.g., see Blomberg et al. 2003; Huey et al. 2003; Rhodes and Kawecki 2009). Specifically, our results demonstrate that selection for a behavioral trait can result in very large changes (in our case, an ~ 3-fold increase in daily running distance; Table S1, Figure 1), without large modifications to underlying morphological or physiological traits (here, only a 13.6% increase in VO_2max (Table 1, Figure 2A), a 6.5% increase in BMR (Table 1, Figure 2C), and no statistically detectable changes in organ masses or hematocrit (Table 2, Figure S1).

Acknowledgements

We thank M.A. Chappell and members of the Garland lab for assistance. We thank P. Koteja for very thorough comments on the manuscript.

Funding

This work was supported by US NSF grants, most recently IOS-2038528.

Tables and Figures

Table 1. VO_2max, BMR, and associated whole-body, lean, and fat mass.

Trait	N	High Runner		Control		Mini-muscle		Normal	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE
VO_2max, 1-min (mL/min)									
Body mass as covariate	72	4.747	(4.65,4.84)	4.179	(4.06,4.30)	4.467	(4.06, 4.30)	4.441	(4.38,4.50)
Lean mass as covariate	72	4.752	(4.61,4.90)	4.269	(4.10,4.44)	4.607	(4.44,4.78)	4.403	(4.32,4.49)
Cooperativity	72	3.629	0.353	3.564	0.421	3.627	0.437	3.566	0.2667
Exhaustion	72	3.261	0.259	3.336	0.346	3.722	0.382	2.874	0.209
Body mass at VO_2max (g)	70	23.49	1.371	24.22	1.429	22.46	1.169	25.25	0.976
Lean mass (g)	70	19.03	1.163	19.05	1.205	17.11	0.969	20.97	0.827
Fat mass (g)	70	2.497	(1.90,3.21)	3.064	(2.31,3.97)	3.459	(2.76,4.27)	2.184	(1.86,2.55)
BMR, 5-min (mL/min)									
Body mass as covariate	69	0.537	(0.49,0.59)	0.504	(0.45,0.56)	0.520	(0.47,0.57)	0.522	(0.49,0.56)
Lean mass as covariate	65	0.544	(0.50,0.60)	0.531	(0.48,0.59)	0.557	(0.51,0.61)	0.519	(0.49,0.55)
Body mass at BMR (g)	71	22.77	1.491	23.27	1.547	21.36	1.250	24.68	1.061
Lean mass (g)	68	18.92	1.153	18.87	1.201	17.12	0.989	20.68	0.819
Fat mass (g)	68	1.879	(1.26,2.68)	2.839	(1.91,4.05)	2.892	(2.05,3.94)	1.839	(1.46,2.28)

Table 1. Continued.

Trait	Linetype			Replicate Line Covariance Parameters			Mini		
	d.f.	F	P	Estimate	SE	P	d.f.	F	P
VO_2max, 1-min (mL/min)									
Body mass as covariate	1,6	87.23	<.0001	0.000015	0.000034	0.328066	1,61	0.16	0.6891
Lean mass as covariate	1,6	31.86	0.0013	0.000066	0.000077	0.195881	1,61	5.07	0.0279
Cooperativity	1,6	0.02	0.9057	0.3791	0.3161	0.115208	1,62	0.02	0.8910
Exhaustion	1,6	0.03	0.8595	0.1469	0.1872	0.216315	1,62	4.22	0.0442
Body mass at VO_2max (g)	1,6	0.14	0.7213	7.1601	4.5019	0.0559	1,61	12.87	0.0007
Lean mass (g)	1,6	0	0.9936	5.1847	3.2186	0.0536	1,61	39.96	<.0001
Fat mass (g)	1,6	1.97	0.2103	0.005871	0.004094	0.0758	1,60	17.71	<.0001
BMR, 5-min (mL/min)									
Body mass as covariate	1,6	2.29	0.1807	0.000342	0.000318	0.140757	1,55	0.01	0.9220
Lean mass as covariate	1,6	0.33	0.5869	0.00026	0.000304	0.196444	1,51	2.59	0.1134
Body mass at BMR (g)	1,6	0.06	0.8203	8.5142	5.3051	0.0543	1,62	17.41	<.0001
Lean mass (g)	1,6	0	0.9799	5.0604	3.1573	0.0545	1,59	28.80	<.0001
Fat mass (g)	1,6	4.1	0.0894	0.009622	0.008182	0.1198	1,57	7.18	0.0096

Least Squares Means (LSM), Standard Errors (SE), F-statistic, and P-values are from SAS Procedure Mixed analyses with linetype and mini-muscle as fixed effects, and line nested within linetype as a random effect. VO_2max and BMR were analyzed with body mass and age as covariates, and separately with lean mass and age as covariates. Fat mass was analyzed with lean mass as a covariate. VO_2max and BMR were $\log_{10}$ transformed to normalize residuals. Fat mass was transformed as $(Fat)^{0.3}$ to normalize residuals. LSMs were back transformed with asymmetrical 95% confidence intervals reported within parentheses in place of SE.

Table 2. Organ masses and associated whole-body, lean, and fat mass.

Trait	N	High Runner		Control		Mini-muscle		Normal	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE
Body mass at dissection (g)	67	24.60	1.464	25.29	1.514	22.84	1.204	27.05	1.041
Lean mass (g)	64	20.36	1.047	20.77	1.095	18.37	0.905	22.76	0.746
Fat mass (g)	64	2.901	(2.20,3.74)	3.463	(2.55,4.58)	3.641	(2.75,4.71)	2.750	(2.33,3.22)
Ventricle (g)	72	0.118	0.003	0.116	0.004	0.118	0.004	0.117	0.002
Triceps surae (g)	72	0.093	0.003	0.090	0.003	0.060	0.003	0.123	0.002
Hematocrit (%)	68	0.455	0.005	0.452	0.007	0.447	0.008	0.451	0.004
Lung (wet) (g)	70	0.233	0.006	0.260	0.011	0.263	0.011	0.229	0.006
Brain (g)	70	0.508	0.013	0.477	0.14	0.493	0.012	0.491	0.009
Liver (g)	72	1.410	0.042	1.459	0.059	1.519	0.052	1.350	0.031
Kidney (g)	72	0.178	0.002	0.175	0.003	0.180	0.003	0.174	0.002
Spleen (g)	72	0.102	0.006	0.098	0.007	0.106	0.007	0.094	0.005

Table 2. Continued.

Trait	Linetype			Replicate Line Covariance Parameters			Mini		
	d.f.	F	P	Estimate	SE	P	d.f.	F	P
Body mass at dissection (g)	1,6	0.11	0.7511	8.2484	5.0978	0.0528	1,58	33.41	<.0001
Lean mass (g)	1,6	0.07	0.7967	4.1262	2.6346	0.0587	1,55	50.03	<.0001
Fat mass (g)	1,6	1.38	0.2845	0.005237	0.006353	0.2049	1,54	3.64	0.0618
Ventricle (g)	1,6	0.27	0.6212	0.000019	0.00002	0.1734	1,61	0.09	0.7670
Triceps surae (g)	1,6	0.76	0.4162	0.000022	0.000022	0.1554	1,61	323.02	<.0001
Hematocrit (%)	1,6	0.13	0.7259	0.000055	0.000079	0.2443	1,58	2.69	0.1061
Lung (wet) (g)	1,6	5.44	0.0585	Non-positive estimate			1,59	7.24	0.0093
Brain (g)	1,6	2.97	0.1354	0.000593	0.000407	0.0723	1,59	0.05	0.8173
Liver (g)	1,6	0.62	0.4616	0.005255	0.004477	0.1202	1,61	10.76	0.0017
Kidney (g)	1,6	0.71	0.4303	Non-positive estimate			1,61	3.20	0.0784
Spleen (g)	1,6	0.19	0.6817	0.000137	0.000106	0.0991	1,61	3.36	0.0716

Least Squares Means (LSM), Standard Errors (SE), F-statistic, and P-values are from SAS Procedure Mixed analyses with linetype and mini-muscle as fixed effects, and line nested within linetype as a random effect. Fat mass was analyzed with lean mass as a covariate. Fat mass was transformed as $(Fat)^{0.3}$ to normalize residuals and LSM were back transformed with asymmetrical 95% confidence intervals reported within parentheses in place of SE. Organ masses were analyzed with body mass and age as covariates. Hematocrit was analyzed with only age as a covariate.

Table 3. Correlations at the level of individual variation.

Individual Mice										
Trait		BMR	Ventricle	Muscle	Hematocrit	Lung	Brain	Liver	Kidney	Spleen
VO_2max	R	0.073	0.285*	0.112	-0.047	0.012	-0.118	-0.021	0.084	-0.114
	P	0.552	0.015	0.347	0.704	0.920	0.332	0.858	0.482	0.341
	N	69	72	72	68	70	70	72	72	72
BMR	R	---	-0.002	0.172	-0.068	0.028	0.104	0.061	-0.084	0.009
	P	---	0.988	0.156	0.589	0.823	0.401	0.619	0.490	0.938
	N	---	69	69	65	67	67	69	69	69
Line Means										
Trait		BMR	Ventricle	Muscle	Hematocrit	Lung	Brain	Liver	Kidney	Spleen
VO_2max	R	0.354	0.140	-0.539	-0.126	-0.349	0.662	0.054	0.565	0.402
	P	0.351	0.720	0.134	0.747	0.357	0.052	0.891	0.113	0.284
	N	9	9	9	9	9	9	9	9	9
BMR	R	---	0.882*	-0.124	0.044	-0.217	0.511	-0.041	0.097	-0.189
	P	---	0.002	0.750	0.911	0.574	0.160	0.917	0.804	0.627
	N	---	9	9	9	9	9	9	9	9

Top (Individual Mice): Correlations using residuals from models in SAS Procedure Mixed with linetype and mini-muscle status as fixed effects, replicate line as a random effect nested within linetype, and body mass [except for hematocrit] and age as covariates, for VO2max and BMR in relation to lower-level traits.
Bottom (Line Means): Correlations using models in SAS Procedure Mixed with line [N=9, separating mini- and normal-muscle mice in Line HR6] as a fixed effect, and body mass [except for hematocrit] and age as covariates, for VO2max and BMR in relation to lower-level traits.

Figure 1. Female sibling wheel-running data on days 5 and 6 of the 6-day trial. Data are presented as untransformed Least Squares Means (LSM) and Standard Errors (SE). HR line 3 is denoted with a slashed bar, as they are fixed for the mini-muscle phenotype. Data were not available for the mini-muscle status of HR line 6 mice used for wheel testing. P-values for A-D are from SAS Procedure Mixed analyses with linetype as a fixed effect and line nested within linetype as a random effect. Wheel freeness and age were used as covariates in each analysis. Full statistical results are in Table S1. (A) Average number of revolutions run on days 5 and 6. (B) Average number of intervals run. (C) Average running speed. (D) Maximum running speed.

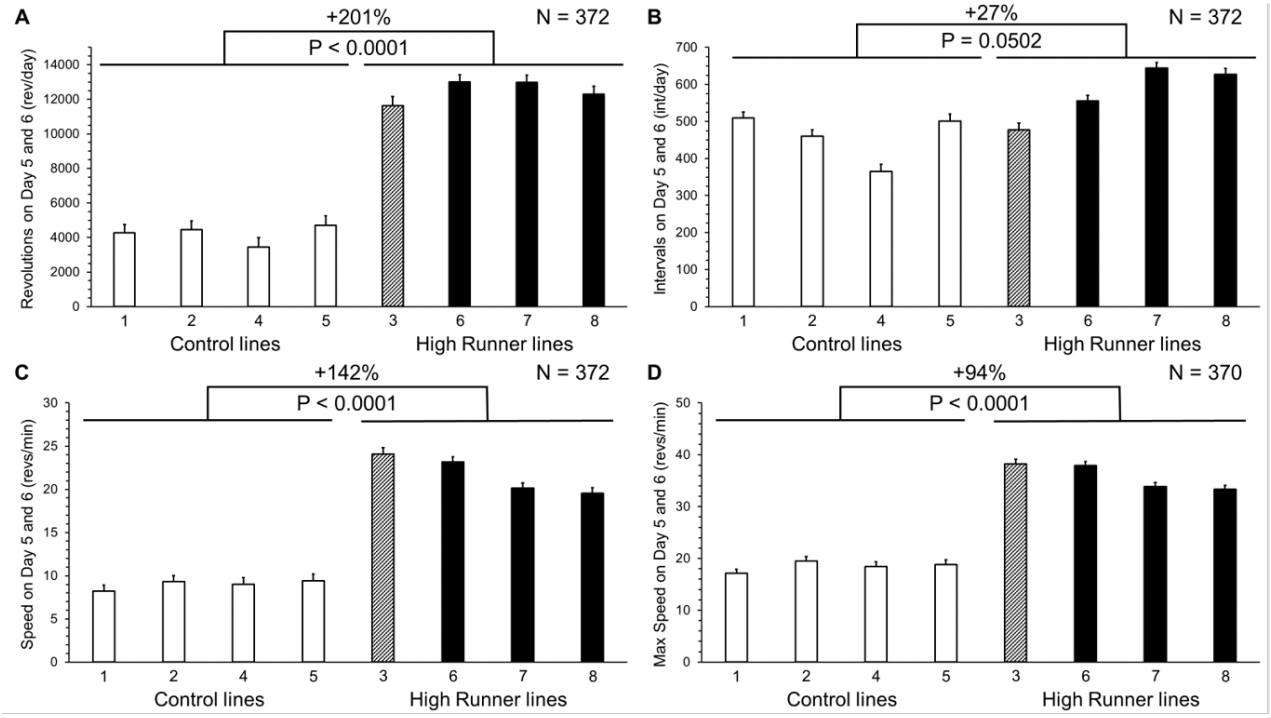
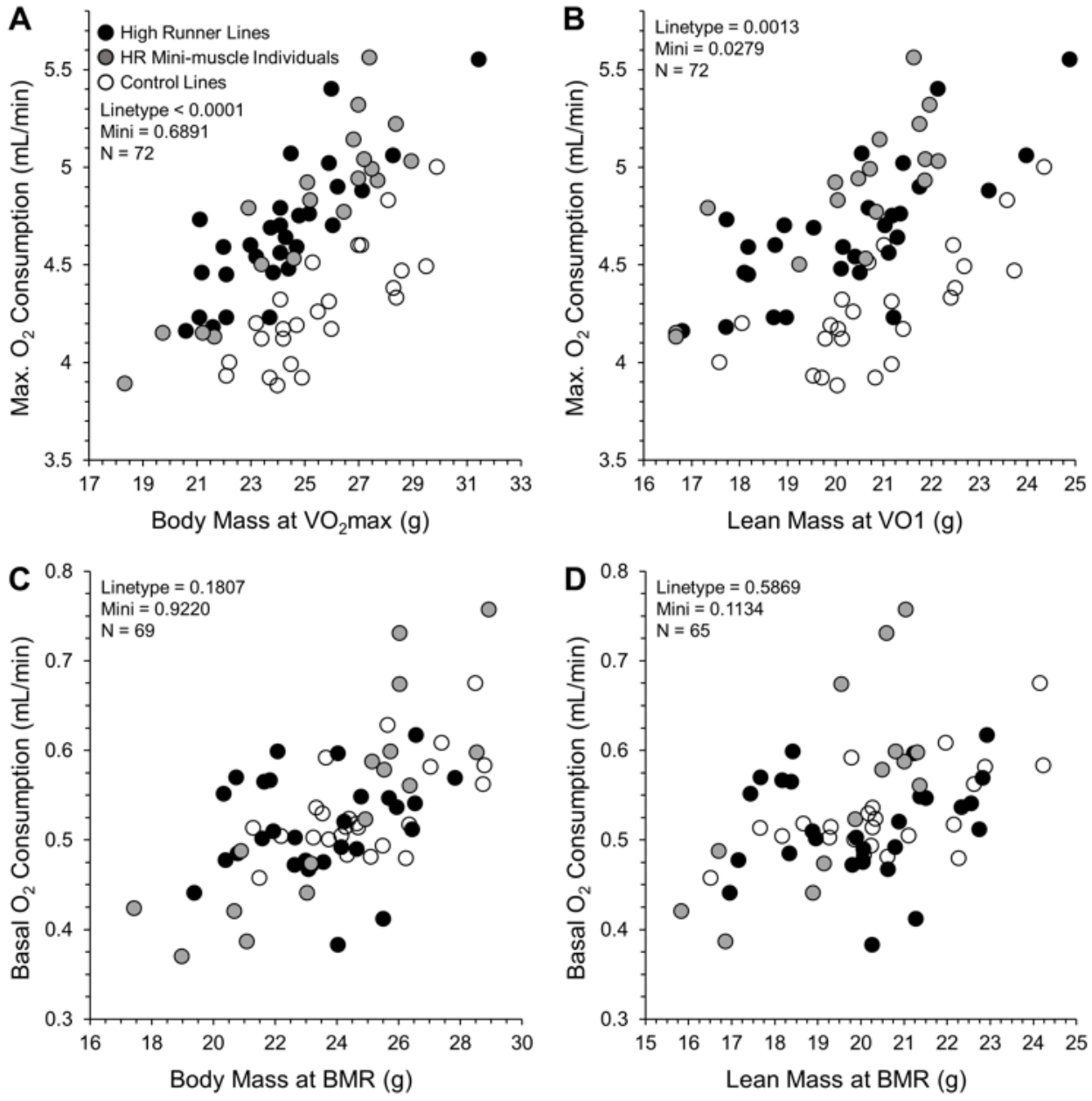


Figure 2. Scatterplot of VO_2max and BMR versus body mass or lean mass. P-values are significance levels from models in SAS Procedure Mixed with linetype and mini-muscle status as fixed effects, replicate line as a random effect nested within linetype, and body (either whole-body or lean) mass and age as covariates.



References

Albuquerque, R. L., G. Sanchez, and T. Garland Jr. 2015. Relationship between maximal oxygen consumption (VO2max) and home range area in mammals. Physiol. Biochem. Zool. 88:660–667.

Andrew, J. R., T. Garland Jr., M. A. Chappell, M. Zhao, and W. Saltzman. 2019. Effects of short- and long-term cold acclimation on morphology, physiology, and exercise performance of California mice (*Peromyscus californicus*): potential modulation by fatherhood. J. Comp. Physiol. B 189:471–487.

Auer, S. K., S. S. Killen, and E. L. Rezende. 2017. Resting vs. active: a meta-analysis of the intra- and inter-specific associations between minimum, sustained, and maximum metabolic rates in vertebrates. Funct. Ecol. 31:1728–1738.

Bartholomew, G. A., D. Vleck, and C. M. Vleck. 1981. Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. J. Exp. Biol. 90:17–32.

Bennett, A. F., and J. A. Ruben. 1979. Endothermy and activity in vertebrates. Science 206:649–654.

Biro, P. A., T. Garland Jr., C. Beckmann, B. Ujvari, F. Thomas, and J. R. Post. 2018. Metabolic scope as a proximate constraint on individual behavioral variation: effects on personality, plasticity, and predictability. Am. Nat. 192:142–154.

Blomberg, S. P., T. Garland Jr., and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. Evolution 57:717–745.

Boratyński, Z. 2020. Energetic constraints on mammalian home-range size. Funct. Ecol. 34:468–474.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology 85:1771–1789.

Cadney, M. D., R. L. Albuquerque, N. E. Schwartz, M. P. McNamara, A. A. Castro, M. P. Schmill, D. A. Hillis, and T. Garland Jr. 2022. Effects of early-life voluntary exercise and fructose on adult activity levels, body composition, aerobic capacity, and organ masses in mice bred for high voluntary wheel-running behavior. J. Dev. Orig. Health Dis. 1–12.

Cadney, M. D., L. Hiramatsu, Z. Thompson, M. Zhao, J. C. Kay, J. M. Singleton, R. L. Albuquerque, M. P. Schmill, W. Saltzman, and T. Garland Jr. 2021. Effects of early-life exposure to Western diet and voluntary exercise on adult activity levels, exercise physiology, and associated traits in selectively bred High Runner mice. Physiol. Behav. 234:113389.

Careau, V., S. Killen, and N. Metcalfe. 2015. Adding fuel to the "fire of life": Energy budgets across levels of variation in ectotherms and endotherms. Pp. 219–234 *in* Integrative Organismal Biology. Wiley-Blackwell.

Careau, V., M. E. Wolak, P. A. Carter, and T. Garland Jr. 2013. Limits to behavioral evolution: The quantitative genetics of a complex trait under directional selection. Evolution 67:3102–3119.

Castro, A. A., F. A. Karakostis, L. E. Copes, H. E. McClendon, A. P. Trivedi, N. E. Schwartz, and T. Garland Jr. 2021. Effects of selective breeding for voluntary exercise, chronic exercise, and their interaction on muscle attachment site morphology in house mice. J. Anat. 240:279–295.

Chappell, M. A., and G. C. Bachman. 1995. Aerobic performance in Belding's Ground Squirrels (*Spermophilus beldingi*): variance, ontogeny, and the aerobic capacity model of endothermy. Physiol. Zool. 68:421–442.

Chappell, M. A., C. Bech, and W. A. Buttemer. 1999. The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. J. Exp. Biol. 202:2269–2279.

Chappell, M. A., and K. A. Hammond. 2004. Maximal aerobic performance of deer mice in combined cold and exercise challenges. J. Comp. Physiol. B 174:41–48.

Claghorn, G. C., Z. Thompson, K. Wi, L. Van, and T. Garland Jr. 2017. Caffeine stimulates voluntary wheel running in mice without increasing aerobic capacity. Physiol. Behav. 170:133–140.

Copes, L., H. Schutz, E. Dlugosz, W. Acosta, M. Chappell, and T. Garland Jr. 2015. Effects of voluntary exercise on spontaneous physical activity and food consumption in mice: Results from an artificial selection experiment. Physiol. Behav. 149:86–94.

Dlugosz, E. M., M. A. Chappell, T. H. Meek, P. Szafrańska, K. Zub, M. Konarzewski, J. H. Jones, E. Bicudo, R. F. Nespolo, V. Careau, and T. Garland Jr. 2013a. Phylogenetic analysis of mammalian maximal oxygen consumption during exercise. J. Exp. Biol. 216:4712–7421.

Dlugosz, E. M., H. Schutz, T. H. Meek, W. Acosta, C. J. Downs, E. G. Platzer, M. A. Chappell, and T. Garland Jr. 2013b. Immune response to a *Trichinella spiralis* infection in house mice from lines selectively bred for high voluntary wheel running. J. Exp. Biol. 216:4212–4221.

Dohm, M. R., J. P. Hayes, and T. Garland Jr. 2001. The quantitative genetics of maximal and basal rates of oxygen consumption in mice. Genetics 159:267–277.

Else, P. L., and A. J. Hulbert. 1981. Comparison of the “mammal machine” and the “reptile machine”: energy production. Am. J. Physiol.-Regul. Integr. Comp. Physiol. 240:R3–R9.

Else, P. L., and A. J. Hulbert. 1987. Evolution of mammalian endothermic metabolism: “leaky” membranes as a source of heat. Am. J. Physiol.-Regul. Integr. Comp. Physiol. 253:R1–R7.

Else, P., N. Turner, and A. Hulbert. 2004. The evolution of endothermy: role for membranes and molecular activity. Physiol. Biochem. Zool. 77:950–958.

Farmer, C. G. 2000. Parental care: The key to understanding endothermy and other convergent features in birds and mammals. Am. Nat. 155:326–334.

Garland Jr., T. 1984. Physiological correlates of locomotory performance in a lizard: An allometric approach. Am. J. Physiol.-Regul. Integr. Comp. Physiol. 247:R806–R815.

Garland Jr., T. 2003. Selection experiments: An under-utilized tool in biomechanics and organismal biology. Pp. 23–56 *in* Vertebrate Biomechanics and Evolution. BIOS Scientific Publishers Limited, Oxford.

Garland Jr., T., and R. L. Albuquerque. 2017. Locomotion, energetics, performance, and behavior: A mammalian perspective on lizards, and vice versa. Integr. Comp. Biol. 57:252–266.

Garland Jr., T., S. A. Kelly, J. L. Malisch, E. M. Kolb, R. M. Hannon, B. K. Keeney, S. L. Van Cleave, and K. M. Middleton. 2011. How to run far: Multiple solutions and sex-specific responses to selective breeding for high voluntary activity levels. Proc. R. Soc. B Biol. Sci. 278:574–581.

Garland Jr., T., M. T. Morgan, J. G. Swallow, J. S. Rhodes, I. Girard, J. G. Belter, and P. A. Carter. 2002. Evolution of a small-muscle polymorphism in lines of house mice selected for high activity levels. Evolution 56:1267–1275.

Gębczyński, A., and M. Konarzewski. 2009. Metabolic correlates of selection on aerobic capacity in laboratory mice: a test of the model for the evolution of endothermy. J. Exp. Biol. 212:2872–2878.

Girard, I., E. Rezende, and T. Garland Jr. 2007. Leptin levels and body composition of mice selectively bred for high voluntary locomotor activity. Physiol. Biochem. Zool. 80:568–579.

Girard, I., J. Swallow, P. Carter, P. Koteja, J. Rhodes, and T. Garland Jr. 2002. Maternal-care behavior and life-history traits in house mice (*Mus domesticus*) artificially selected for high voluntary wheel-running activity. Behav. Processes 57:37–50.

Hayes, J. P., and T. Garland Jr. 1995. The evolution of endothermy: Testing the aerobic capacity model. Evolution 49:836–847.

Hillman, S., T. Hancock, and M. Hedrick. 2013. A comparative meta-analysis of maximal aerobic metabolism of vertebrates: implications for respiratory and cardiovascular limits to gas exchange. J Comp Physiol B 183:167–179.

Hiramatsu, L., and T. Garland Jr. 2018. Mice selectively bred for high voluntary wheel-running behavior conserve more fat despite increased exercise. Physiol. Behav. 194:1–8.

Hiramatsu, L., J. Kay, Z. Thompson, J. Singleton, G. Claghorn, R. Albuquerque, B. Ho, B. Ho, G. Sanchez, and T. Garland Jr. 2017. Maternal exposure to Western diet affects adult body composition and voluntary wheel running in a genotype-specific manner in mice. Physiol. Behav. 179:235–245.

Houle-Leroy, P., H. Guderley, J. G. Swallow, and T. Garland Jr. 2003. Artificial selection for high activity favors mighty mini-muscles in house mice. Am. J. Physiol.-Regul. Integr. Comp. Physiol. 284:R433–R443.

Huey, R. B., P. E. Hertz, and B. Sinervo. 2003. Behavioral drive versus behavioral inertia in evolution: a null model approach. Am. Nat. 161:357–366.

Hulbert, A. J., and P. L. Else. 2004. Basal metabolic rate: history, composition, regulation, and usefulness. Physiol. Biochem. Zool. 77:869–876.

Hulbert, A. J., and P. L. Else. 1989. Evolution of mammalian endothermic metabolism: mitochondrial activity and cell composition. Am. J. Physiol.-Regul. Integr. Comp. Physiol. 256:R63–R69.

Hulbert, A. J., N. Turner, J. Hinde, P. L. Else, and H. Guderley. 2006. How might you compare mitochondria from different tissues and different species? J. Comp. Physiol. B 176:93–105.

Jensen, T. L., M. K. Kiersgaard, D. B. Sørensen, and L. F. Mikkelsen. 2013. Fasting of mice: A review. Lab. Anim. 47:225–240.

Kane, S. L., T. Garland Jr., and P. A. Carter. 2008. Basal metabolic rate of aged mice is affected by random genetic drift but not by selective breeding for high early-age locomotor activity or chronic wheel access. Physiol. Biochem. Zool. 81:288–300.

Keeney, B. K. 2011. Behavioral, neural, and life history correlates of selective breeding for high voluntary exercise in house mice. University of California, Riverside.

Kelly, S. A., T. A. Bell, S. R. Selitsky, R. J. Buus, K. Hua, G. M. Weinstock, T. Garland Jr., F. Pardo-Manuel de Villena, and D. Pomp. 2013. A novel intronic single nucleotide polymorphism in the Myosin heavy polypeptide 4 gene is responsible for the mini-muscle phenotype characterized by major reduction in hind-limb muscle mass in mice. Genetics 195:1385–1395.

Kelly, S., F. Gomes, E. Kolb, J. Malisch, and T. Garland Jr. 2017. Effects of activity, genetic selection and their interaction on muscle metabolic capacities and organ masses in mice. J. Exp. Biol. 220:1038–1047.

Kolb, E. M., S. A. Kelly, K. M. Middleton, L. S. Sermsakdi, M. A. Chappell, and T. Garland Jr. 2010. Erythropoietin elevates VO2max but not voluntary wheel running in mice. J. Exp. Biol. 213:510–519.

Konarzewski, M., and J. Diamond. 1995. Evolution of basal metabolic rate and organ masses in laboratory mice. Evolution 49:1239–1248.

Konarzewski, M., and A. Książek. 2013. Determinants of intra-specific variation in basal metabolic rate. J. Comp. Physiol. B 183:27–41.

Koteja, P. 2000. Energy assimilation, parental care and the evolution of endothermy. Proc. R. Soc. Lond. B Biol. Sci. 267:479–484.

Koteja, P., and T. Garland Jr. 2001. Response to R. Eikelboom. Anim. Behav. 61:F25-26.

Książek, A., M. Konarzewski, and I. B. Łapo. 2004. Anatomic and energetic correlates of divergent selection for basal metabolic rate in laboratory mice. Physiol. Biochem. Zool. 77:890–899.

Lacy, R. C., and C. B. Lynch. 1979. Quantitative genetic analysis of temperature regulation in *Mus musculus*. I. Partitioning of variance. Genetics 91:743–753.

Lovegrove, B. 2019. Fires of life: Endothermy in birds and mammals. Yale University Press, New Haven ; London.

Malisch, J., C. Breuner, E. Kolb, H. Wada, R. Hannon, M. Chappell, K. Middleton, and T. Garland Jr. 2009. Behavioral despair and home-cage activity in mice with chronically elevated baseline corticosterone concentrations. Behav. Genet. 39:192–201.

McNamara, M. P., M. D. Cadney, A. A. Castro, D. A. Hillis, K. M. Kallini, J. C. Macbeth, M. P. Schmill, N. E. Schwartz, A. Hsiao, and T. Garland Jr. 2022. Oral antibiotics reduce voluntary exercise behavior in athletic mice. Behav. Processes 199:104650.

Nespolo, R. F., J. J. Solano-Iguaran, and F. Bozinovic. 2017. Phylogenetic analysis supports the aerobic-capacity model for the evolution of endothermy. Am. Nat. 189:13–27.

Pough, F. H., and R. M. Andrews. 1984. Individual and sibling-group variation in metabolism of lizards: The aerobic capacity model for the origin of endothermy. Comp. Biochem. Physiol. A Physiol. 79:415–419.

Rezende, E., F. Gomes, M. Chappell, and T. Garland Jr. 2009. Running behavior and its energy cost in mice selectively bred for high voluntary locomotor activity. Physiol. Biochem. Zool. 82:662–679.

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

Rezende, E. L., T. Garland Jr., M. A. Chappell, J. L. Malisch, and F. R. Gomes. 2006a. Maximum aerobic performance in lines of *Mus* selected for high wheel-running activity: effects of selection, oxygen availability and the mini-muscle phenotype. J. Exp. Biol. 209:115–127.

Rezende, E. L., F. R. Gomes, J. L. Malisch, M. A. Chappell, and T. Garland Jr. 2006b. Maximal oxygen consumption in relation to subordinate traits in lines of house mice selectively bred for high voluntary wheel running. J. Appl. Physiol. 101:477–485.

Rhodes, J. S., and T. J. Kawecki. 2009. Behavior and neurobiology. Pp. 263–300 *in* T. Garland Jr. and M. R. Rose, eds. Experimental evolution: Concepts, methods, and applications of selection experiments. University of California Press.

Sadowska, E. T., M. K. Labocha, K. Baliga, A. Stanisz, A. K. Wróblewska, W. Jagusiak, and P. Koteja. 2005. Genetic correlations between basal and maximum metabolic rates in a wild rodent: Consequences for evolution of endothermy. Evolution 59:672–681.

Sadowska, E. T., C. Stawski, A. Rudolf, G. Dheyongera, K. M. Chrząścik, K. Baliga-Klimczyk, and P. Koteja. 2015. Evolution of basal metabolic rate in bank voles from a multidirectional selection experiment. Proc. R. Soc. B Biol. Sci. 282:20150025.

Singleton, J., and T. Garland Jr. 2019. Influence of corticosterone on growth, home-cage activity, wheel running, and aerobic capacity in house mice selectively bred for high voluntary wheel-running behavior. Physiol. Behav. 198:27–41.

Storz, J. F., J. T. Bridgham, S. A. Kelly, and T. Garland Jr. 2015. Genetic approaches in comparative and evolutionary physiology. Am. J. Physiol.-Regul. Integr. Comp. Physiol. 309:R197–R214.

Swallow, J. G., P. A. Carter, and T. Garland Jr. 1998a. Artificial selection for increased wheel-running behavior in house mice. Behav. Genet. 28:227–237.

Swallow, J. G., and T. Garland Jr. 2005. Selection experiments as a tool in evolutionary and comparative physiology: Insights into complex traits. Integr. Comp. Biol. 45:387–390.

Swallow, J. G., T. Garland Jr., P. A. Carter, W.-Z. Zhan, and G. C. Sieck. 1998b. Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*). J. Appl. Physiol. 84:69–76.

Swallow, J. G., J. P. Hayes, P. Koteja, and T. Garland Jr. 2009a. Experimental evolution: Concepts, methods, and applications of selection experiments. P. *in* T. Garland Jr. and M. R. Rose, eds. Experimental evolution: Concepts, methods, and applications of selection experiments. University of California Press.

Swallow, J. G., J. P. Hayes, P. Koteja, and T. Garland Jr. 2009b. Experimental evolution: Concepts, methods, and applications of selection experiments. P. *in* T. Garland Jr. and M. R. Rose, eds. Selection experiments and experimental evolution of performance and physiology. University of California Press.

Taigen, T. L. 1983. Activity metabolism of Anuran amphibians: Implications for the origin of endothermy. Am. Nat. 121:94–109.

Wallace, I. J., and T. Garland Jr. 2016. Mobility as an emergent property of biological organization: Insights from experimental evolution. Evol. Anthropol. 25:98–104.

Weibel, E., L. Bacigalupe, B. Schmitt, and H. Hoppeler. 2004. Allometric scaling of maximal metabolic rate in mammals: muscle aerobic capacity as determinant factor. Respir. Physiol. Neurobiol. 140:115–132.

Wone, B. W. M., P. Madsen, E. R. Donovan, M. K. Labocha, M. W. Sears, C. J. Downs, D. A. Sorensen, and J. P. Hayes. 2015. A strong response to selection on mass-independent

maximal metabolic rate without a correlated response in basal metabolic rate. Heredity 114:419–427.

Wu, N. C., and F. Seebacher. 2022. Physiology can predict animal activity, exploration, and dispersal. Commun. Biol. 5:1–11.