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Effects of Genetic Background and Early-Life Exercise on Adult Traits Related to Exercise Behavior, Morphology, and Physiology

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

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in

Evolution, Ecology, and Organismal Biology

by

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ABSTRACT OF THE DISSERTATION

Effects of Genetic Background and Early-Life Exercise on Adult Traits Related to Exercise Behavior, Morphology, and Physiology

by

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Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, September 2023
Dr. Theodore Garland Jr., Chairperson

I studied correlated responses to artificial selection for high voluntary wheel-running behavior. Chapter 1 used meta-analysis to summarize comparisons of four replicate High Runner (HR) lines with four non-selected Control (C) lines. Effect sizes from 34 studies demonstrate that HR mice have evolved larger hearts and smaller bodies, and that plateaus in effect sizes for both traits coincide with selection limits for wheel running.

Chapter 2 tested whether HR mice would have a higher maximal rate of oxygen consumption during forced exercise (VO_2max), a higher basal metabolic rate (BMR), and increases in the size of relevant organs. Although HR mice ran ~3-fold more revolutions/day and had higher VO_2max, they did not have a statistically higher BMR nor differ in relative organ masses (heart, lung, liver, kidney, spleen, brain, calf muscle) or hematocrit. Thus, a large evolutionary increase in activity level has not required proportionally large changes in underlying morphological or physiological traits.

Chapter 3 examined effects of maternal exercise on maternal care and offspring body composition and activity behavior. HR females ran more than C prior to breeding and before parturition, but not after. When housed with wheels, HR females had reduced maternal care compared to C during days 1-10 after parturition. HR females with wheels had fewer litters survive to weaning than those without wheels, but the opposite occurred for C females. For both HR and C dams with wheels, surviving offspring had delayed development as indicated by eye opening and lean mass at weaning. However, maternal wheel access did not alter offspring adult body mass, activity behavior or reproductive success.

Overall, the evolution of high voluntary exercise did not require large changes in underlying physiology (e.g., VO_2max, BMR), but morphological traits (e.g., body mass, relative heart mass) may have imposed constraints on the evolution of activity levels in these mice. Moreover, HR mothers differ in behavior and/or resource allocation resources when given the opportunity to

exercise, which results in costs to offspring survival. Further research is needed to determine the underlying mechanisms of this apparent trade-off between two important biological drives.

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Introduction

I am interested in understanding how behavior evolves in a correlated fashion with traits at other levels in the phenotypic hierarchy, including life history, whole-organism performance, and lower-level (subordinate) traits. As a result, the scope of my dissertation is somewhat broad, and so each chapter will have its own introduction where I provide background information and elaborate on the conceptual framework of the topic therein. However, my dissertation also represents the sum of my educational and professional development over the past 6 years. So here, I will comment on some topics that have shaped how I have conducted and interpreted my research.

Since 1982, organismal biologists have developed a broad framework to think about performance, behavior, and other complex traits. The Morphology-Performance-Fitness Paradigm (MPF) describes how selection may act most directly on traits strongly correlated with Darwinian fitness (e.g., whole-organism performance), whereas its influence is weaker on lower-level traits (e.g., morphology) (Arnold 1983). The path analysis introduced by Arnold also describes how lower-level traits might directly affect higher levels of biological organization, specifically performance abilities. However, Arnold did not recognize behavior as a distinct level of biological organization in his model; rather, he used “morphology as shorthand for any measurable or countable aspect of structure, physiology, or behavior” (Arnold 1983 p. 348). Many studies have since expanded on Arnold’s original ideas (Kingsolver and Huey 2003;

Garland Jr. and Kelly 2006; Lailvaux and Husak 2014; Storz et al. 2015; Dantzer et al. 2016; Garland Jr. and Albuquerque 2017; Higham et al. 2021), including for plants (Ackerly et al. 2000; Violle et al. 2007), and Behavior was later added to emphasize its potential as a filter between selection and performance abilities (Garland Jr. and Carter 1994; Garland Jr. and Losos 1994). For example, animals confronted with a predator might remain motionless, rather than fleeing at top speed, and such a behavior could "shield" locomotor performance from the direct effects of selection (Garland Jr. et al. 1990).

Selection experiments can be used to elucidate both the evolutionary and biological underpinnings of complex organismal traits (e.g., exercise behavior). By studying responses to selection on complex traits, one may investigate potential links between sub-organismal characteristics that influence performance (Rhodes and Kawecki 2009; Swallow et al. 2009). For my dissertation research, I utilized mice from a long-term artificial selection experiment for high levels of voluntary wheel running. Briefly (as information can also be found in the chapters below), the four replicate High Runner (HR) lines are bred for high voluntary wheel-running behavior and compared with four replicate non-selected Control (C) lines (Swallow et al. 1998; Garland Jr. 2003; Careau et al. 2013; Wallace and Garland Jr. 2016). For the four HR lines, the highest-running male and female from each family are chosen as breeders for the next generation (no sibling pairs). For the four replicate C lines, breeders are chosen without regard to wheel running. Selection for high levels of voluntary

wheel running, an energetically demanding behavior (Koteja et al. 1999; Swallow et al. 2001; Rezende et al. 2009; Copes et al. 2015), is likely to influence numerous aspects of their biology. As expected, many adult traits differ between HR and C lines (Rhodes et al. 2005; Garland Jr. et al. 2011; Wallace and Garland Jr. 2016), including increased home-cage activity (spontaneous physical activity) when housed without access to wheels (Malisch et al. 2009; Copes et al. 2015), increased maximal oxygen consumption (VO_2max) (Rezende et al. 2005; Kolb et al. 2010; Schwartz et al. 2023), brain size (Kolb et al. 2013; Schmill et al. 2023), reduced body fat (Swallow et al. 2001; Girard et al. 2007), and altered circulating levels of hormones (Girard et al. 2007; Malisch et al. 2007; Vaanholt et al. 2007; Garland Jr. et al. 2016).

I was particularly interested in working with this mouse model (it was even a major factor in deciding to study at UCR), as selection has been applied to a voluntary behavior. Most voluntary behaviors can be classified as complex traits, and physical activity is no exception (Swallow and Garland Jr. 2005; Garland Jr. and Kelly 2006; Garland Jr. et al. 2017; Lightfoot et al. 2018). The degree of engagement in physical activity is determined by both motivation and physical abilities. In turn, both motivation and ability involve multiple components, and a multitude of sub-organismal traits, each of which are potentially influenced by both genetic and environmental factors. Additionally, many of the subordinate traits may interact in non-additive ways (e.g., see Figure 1 in Garland Jr. and Kelly 2006; Figure 1 in Storz et al. 2015; Figure 5 in Hiramatsu and Garland Jr.

2018), further adding to the complexity. As a result, despite the importance of physical activity in preventing disease and promoting physical and mental health (Mokdad et al. 2004; Haskell et al. 2007; Mikkelsen et al. 2017), the factors influencing individual differences in exercise behavior are poorly understood (Lightfoot et al. 2018).

Phenotypic variation among adults within a population is caused by both genetic and environmental factors. Genetic influences on physical activity, such as voluntary exercise, have been demonstrated in humans (Rowland 2016) and animal models such as laboratory rats and mice (Lightfoot et al. 2018). A number of clear environmental factors have also been established, such as aspects of the "built environment" (e.g., presence of safe parks), that influence how likely people are to exercise (Sallis et al. 2012). Similarly, physical education and sports participation in school can play a large role in the overall activity and energy budgets of children and adolescents.

Some environmental factors may act early in life, yet have long-lasting effects on adult traits. Such early-life experiences, particularly if they occur during critical periods of development and growth, can even affect adult health (Barker 2007; Hanson and Gluckman 2008; Garland Jr. et al. 2017). However, early-life developmental "programming" might be beneficial or detrimental, depending on whether it tends to increase or decrease health-related traits (e.g., physical activity, body fat). For example, various studies demonstrate that human adult body composition can be partially "programmed" by developmental

experiences (Vohr and McGarvey 1997; Hammami et al. 2001; Sayer and Cooper 2005; Haugen et al. 2014), leading to a higher risk for several maladies (e.g., obesity, cardiovascular disease, Type II diabetes) (Curhan et al. 1996b,a; Wei et al. 2003; Allcock et al. 2009; Yu et al. 2011; Johnsson et al. 2014; Mamun et al. 2014; Skilton et al. 2014; Gaillard 2015; Chiavaroli et al. 2016; Godfrey et al. 2017). With regard to physical activity, early-life malnutrition has been shown to affect adult levels of physical activity and related traits, as seen in the aftermath of the Dutch famine (Stein et al. 2009). Rodent models have demonstrated positive effects of early-life exposure to voluntary exercise (running wheels), where mice exposed to wheels from weaning to sexual maturity, then given a washout period (no wheels) of several weeks (equivalent to several years in humans), had increased adult voluntary exercise (Acosta et al. 2015; Cadney et al. 2022). Rodent and human studies have also suggested that parental exercise, particular maternal exercise (Mattocks et al. 2008; Eclarinal et al. 2016) (but also see McPherson et al. 2015; and Short et al. 2017 for examples of paternal exercise effects), may have a positive effect on the physical activity of offspring. Specifically, Mattocks et al. (2008) found that in humans, parents' physical activity during pregnancy and early in life showed a modest associated with the physical activity of their children at 11-12 years of age. Eclarinal et al. (2016) found that maternal exercise before and during pregnancy in adult female mice increased offspring physical activity as adults. However, the mechanisms

underlying these effects are poorly understood (Andersen et al. 2009; Ridgway et al. 2011) and these results are not always present (e.g., see Kelly et al. 2015).

My dissertation is comprised of three chapters whose collective goal is to help elucidate the effects of both early-life exercise and genetic background on adult traits related to exercise behavior, physiology, and morphology. In Chapter 1, I used meta-analytic techniques to summarize and analyze two traits related to exercise behavior (body mass and relative heart ventricle mass) from published and unpublished comparisons of HR and C mice. In Chapter 2, I examined maximal and basal metabolic rates (two key boundaries of animal energetics) in HR and C mice to test a long-standing model for the evolution of vertebrate energetics (i.e., the Aerobic Capacity model). In Chapter 3, I examined the effects of maternal exercise on offspring body composition and activity behavior, and the potential role of exercise as a competing biological “drive” (e.g., see Stults-Kolehmainen 2023) with maternal care behavior (e.g., frequency of nursing).

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1. A meta-analysis of whole-body and heart mass effect sizes from a long-term artificial selection experiment for high voluntary exercise

Abstract

Effect sizes are a quantitative measure of the magnitude and direction of a phenomenon. These estimates can be compiled across multiple studies to summarize the weight of evidence for a particular alternative hypothesis, which is generally termed a meta-analysis. A meta-analytic approach allows one to summarize information from a wide array of similar studies and reach conclusions that may not be apparent on a case-by-case basis (e.g., to determine the overall effectiveness of a drug). Meta-analyses may be particularly useful in selection experiments, where the magnitude of a correlated response is necessarily expected to increase across generations, perhaps eventually reaching a plateau. Here, we examined the effect of artificial selection for high voluntary wheel running on heart mass in mice, which was expected to increase with selection for aerobic exercise behavior. The experiment spans nearly 30 years and 100 generations. All 4 of the replicate High Runner (HR) lines reached selection limits around generations 17-27, running approximately 2.5-3-fold more revolutions per day than the 4 non-selected Control (C) lines. These selection limits cannot be explained simply by a complete loss of heritability. Functional studies have tried to elucidate subordinate traits that affect motivational and/or ability for endurance exercise and have shown that HR

mice differ from C in several other behavioral, physiological, and morphological traits. Several studies of heart ventricle mass have reported larger values in HR mice (with body mass as a covariate), but the differences often do not reach statistical significance ($P<0.05$). We compiled Least-Square Means, standard errors, F-statistics, degrees of freedom, and p-values from multiple studies. We also recorded pertinent information, such as: covariates used, age, generation, and whether other main effects were present (e.g., sex, wheel access). We calculated a common measure of effect size (Pearson's R) and associated confidence intervals. This work is part of a larger meta-analysis to quantify effects of selection on various traits across multiple levels of biological organization. Preliminary results indicate that, despite a lack of statistical significance in many generations, HR mice have evolved larger hearts and smaller bodies relative to Controls. Moreover, a plateau in effect sizes for both of these traits coincide with the generational range during which the selection limit for wheel-running behavior was reached.

Introduction

Meta-analysis dates to 1904, when Karl Pearson collected correlation coefficients to determine the degree to which smallpox inoculation saved lives (Pearson 1904; Rosenthal and Rosnow 2008). Later, Fisher (1948) developed a method for combining the p-values of several independent tests to draw conclusions regarding an overall hypothesis. However, the term meta-analysis was not coined until 1976 (by Gene Glass), referring to “the statistical analysis of a large collection of results from individual studies for the purpose of integrating their findings” (Glass 1976). Now, large sample sizes can be easily gathered through readily available literature, and meta-analysis provides the opportunity for researchers to collect, integrate, and strengthen conclusions based on their own and other studies (Rosenthal and Rosnow 2008; Goh et al. 2016).

The p-value, though an efficient tool for evaluating statistical significance at a glance, may not always be best for determining the overall effect of a treatment (that is, the "effect size") based on multiple studies (Rosenthal and Rosnow 2008; Halsey et al. 2015; Goh et al. 2016; Halsey 2019). For example, what conclusion should be drawn from three studies with a similar directional trend, but reporting p-values of 0.05, 0.06, and 0.20? This is particularly troublesome when the effect being detected is relatively small, sample sizes are limited, and/or studies lack statistical power (Cohen 1988). Instead, meta-analyses redirect the focus to overall effect sizes and a collective value for statistical significance. Additionally, meta-analyses allow one to describe the

variability of effect sizes and the nature of factors that may predict their relative magnitude (i.e., "moderator variables") , thus further clarifying the “big picture” while leveraging the statistical power provided by a relatively larger sample size (Rosenthal and Rosnow 2008; Goh et al. 2016).

Meta-analyses have been routinely used within biomedical fields, notably for their ability to aggregate the results of several independent clinical studies to promote a conclusive answer as to the effectiveness of a treatment. Although infrequently used at first, meta-analyses are now starting to be more commonly used in other fields, similarly for their ability to collate multiple independent studies and provide a more definitive answer regarding an effect of some kind. One application for which meta-analyses seem particularly well-suited is summarizing and synthesizing the results of selection experiments and experimental evolution (Garland and Rose 2009). For example, a meta-analytic approach allows one to summarize important information from an array of similar studies and reach conclusions that may not be apparent on a case-by-case basis; especially useful where the magnitude of a correlated response is expected to increase across generation, perhaps eventually reaching a plateau.

Here, we apply meta-analytic techniques to summarize some key traits from a long-term artificial selection experiment for high voluntary wheel-running behavior. Starting in 1993, a founding population of 224 outbred Hsd:ICR strain (Hauschka and Mirand 1973) house mice (*Mus domesticus*) was used to establish 8 closed lines. Four lines were designated as Control (C) and four as

High Runner (HR). The four replicate HR lines have been bred for voluntary activity on wheels, an energetically demanding behavior (Koteja et al. 1999; Swallow et al. 2001; Rezende et al. 2009; Copes et al. 2015) that involves all organ systems. As expected, many adult traits related to exercise ability and/or motivation have been shown to differ between the HR and the non-selected C lines. However, some apparent differences between HR and C lines have not proven statistically significant in all generations (e.g., see Castro et al. 2021). This raises an important question: are results truly non-significant or are they a product of limited power and Type II errors?

The HR mouse selection experiment is ongoing (100+ generations) and has an extensive publication history (>160 papers). Although individual studies may lack sufficient power to detect small effect sizes, a sample size this large and the additional power provided by meta-analytic procedures allows for detection of even small effect sizes. Additionally, each publication uses similar methodology (a benefit of a single lab conducting repeated measurements of traits) and a near-identical statistical model for analyses. Overall, these make the HR mouse model an excellent candidate for meta-analysis.

To illustrate the procedures, we apply meta-analysis to body mass and heart ventricle mass (with body mass as a covariate), two traits that have been measured repeatedly and for which statistical significance has varied. The present study is intended to be part of a broader synthesis of traits related to

voluntary exercise, across multiple levels of biological organization, that have been studied in the HR mouse model.

Methods

The High Runner mouse selection experiment

The HR mouse model is a long-term artificial selection experiment for high voluntary wheel-running behavior in laboratory house mice (*Mus domesticus*), and has been ongoing since 1993 (now over 100+ generations) (Swallow et al. 1998; Garland Jr. 2003; Garland Jr. et al. 2011b). Each generation has followed a standard protocol as follows: (1) mice are weaned at 21 days of age; (2) housed four per cage from weaning until sexual maturity; (3) housed individually with access to an exercise wheel (1.12-m circumference) for 6 days beginning at ~6-8 weeks of age; (4) for the 4 replicate HR lines, the highest-running male and female from each family are chosen as breeders for the next generation (within-family selection; no sibling pairings allowed), whereas in the 4 replicate C lines, breeders are chosen without regard to wheel running; (5) males and females are co-housed for 18-days; (6) males are removed from cages on the 19th day; (7) offspring births occur, typically over the span of 1 week, but sometimes longer; (8) offspring are weaned at 21 days of age.

The statistical model used for analyses relatively unchanged. Generally, mixed models are implemented using SAS Proc MIXED (SAS Institute, Cary, NC, USA) with restricted maximum likelihood (REML) estimation. Linetype is a fixed

effect and replicate line (4 HR and 4 C) is nested within linetype as a random effect using the containment method for d.f., such that the d.f. for linetype are always 1 and 6. When present, mini-muscle status (an autosomal, Mendelian recessive, single nucleotide polymorphism that results in ~50% reduction in muscle mass along with many other pleiotropic effects (Garland Jr. et al. 2002; Houle-Leroy et al. 2003; Kelly et al. 2013; Copes et al. 2015; Castro et al. 2021; Schwartz et al. 2023)) is an additional fixed effect, tested relative to the residual d.f. Covariates are used as appropriate.

Criteria for inclusion in meta-analysis

The HR selection experiment has an extensive publication history (available at: https://sites.google.com/ucr.edu/hrmice/publications). Unpublished studies, including the dissertations of former graduate students, were also available. Data sets were included if: (1) both body and heart (ventricle) mass were available, (2) all 8 lines were sampled, and (3) a non-experimental linetype group (e.g., HR and C mice without wheels) was included. Implementation of these criteria resulted in 34 unique sets of data. Some studies included both males and females, and were subsequently split by sex, resulting in 48 total effect sizes for each trait (25 for females, 23 for males). These were subdivided into two groups, data from before the selection limit for voluntary wheel-running behavior (~ generation 30, as per Careau et al. 2013), and data from after the limit. Thus, we had 24 estimates of effect sizes (per trait) from before generation

30 (12 for females, 12 for males) and 24 from after generation 30 (13 for females, 11 for males).

Statistical analyses

Data gathered for the present study (such as age, sex, and sample size) are reported in Table 1.1. Least Squares Means (LSMs), Standard Errors (SE), F-statistics, and p-values were recorded. When these values were not available (e.g., unpublished studies), the original data were analyzed in SAS Proc MIXED to generate the necessary values. Fisher's combined probability test (also referred to as Fisher's method) (Mosteller and Fisher 1948) was used to determine an overall p-value for body mass and relative heart mass, using the following formula:

$$\chi^2 = -2 \sum_{i=1}^{k} \ln (p_i)$$

The resulting χ^2 is a cumulative test statistic, k is the number of studies, and p_i is the p-value from each of the independent studies. The χ^2, with 2k degrees of freedom, can subsequently be used to calculate an overall p-value. The F-statistic and degrees of freedom were used to calculate an effect size estimate (in this case, Pearson's r) by using the following formula:

$$r = \sqrt{\frac{F}{F + d.f.}}$$

As noted above, d.f. for comparison of the HR and C lines were always 1 and 6. Therefore, r and the original p-values are directly, although not linearly, related (Figure 1.1). A positive effect size estimate indicates that HR mice have a larger value than C mice, whereas a negative estimate indicates that HR mice have a smaller value for a given trait. Effect sizes were analyzed using an ANCOVA in SPSS v.28, with sex as a fixed effect, and with generation and age as covariates. Outliers were removed if the standardized residual was greater than 3 standard deviations and/or the difference from the next value was greater than ~1 standard deviation (N = 2).

New data for body and heart ventricle mass

New data were collected to provide information on HR mice at a more recent point in the selection experiment. Retired male breeders (N = 148) were used from generation 97 of the selection experiment. These males were euthanized via CO_2 immediately following their 18-day breeding period (as described above). Mice were then weighed and dissected to determine heart ventricle mass. Retired female breeders (N = 95) from generation 99 were also sampled. These females were euthanized via CO_2 after offspring were weaned (21 days post-partum) and dissected immediately.

Results

Body mass before the selection limit

Before the selection limit, the average difference in body mass LSMs between HR and C was 1.08 g for females (HR females: 29.75 ± 2.36; C females: 30.82g ± 1.76) and 1.09 g for males (HR males: 35.15 g ± 2.35; C males: 36.23 g ± 1.86) (Table 1.2, Figures 1.2A and 1.2B). Only 4 of 24 measurements reported a statistically significant ($P < 0.05$) difference in body mass between HR and C lines, all in later generations (Table 1.2, Figure 1.2C). ANCOVA indicated that body mass effect size decreased across generations ($P_{Generation} < 0.0001$, Table 1.2, 2D), with no effect of sex ($P_{Sex} = 0.8718$), no sex*generation interaction ($P_{Generation*Sex} = 0.8963$), and older mice had more positive effect sizes (i.e., magnitude of effect size decreased) ($P_{Age} = 0.0225$).

Body mass after the selection limit

After the selection limit, the average difference in body mass LSMs between HR and C was 2.76 g for females (HR females:26.56 g ± 3.74; C females: 29.32 g ± 4.11) and 3.41 g for males (HR males:30.74 g ± 2.54; C males: 34.15 g ± 3.47) (Table 1.2, Figures 1.2A and 1.2B). Only 8 of 22 measurements reported a statistically significant ($P < 0.05$) difference in body mass at dissection between HR and C lines (Table 1.2, Figure 1.2C). ANCOVA indicated that body mass showed a sex*generation interaction ($P_{Generation*Sex} =$

0.0391, Table 1.2, Figure 1.2D), where effect size was smaller in females across generations and larger for males (age was not significant, $P_{Age} = 0.7716$).

Heart mass before the selection limit

Before the selection limit, the average difference in heart mass LSMs (from analyses with body mass as a covariate) between HR and C was 2.29 mg for females (HR females: 125.71 mg ± 8.78; C females: 123.42 mg ± 6.90) and 2.29 mg for males (HR males: 146.70 mg ± 6.93; C males: 144.41 mg ± 7.23) (Table 1.2, Figures 1.3A and 1.3B). None of the 24 measurements indicated a statistically significant ($P < 0.05$) difference between HR and C lines (Table 1.2, Figure 1.3C). ANCOVA indicated that heart mass effect size increased across generations ($P_{Generation} = 0.0156$, Table 1.2, Figure 1.3D), with no effect of sex ($P_{Sex} = 0.8833$), no sex*generation interaction ($P_{Generation*Sex} = 0.4290$, Table 1.2), and no effect of age ($P_{Age} = 0.1674$).

Heart mass after the selection limit

After the selection limit, the average difference in heart mass LSMs (from analyses with body mass as a covariate) between HR and C 7.40 mg for females (HR females: 134.86 mg ± 14.83; C females: 127.46 mg ± 15.57) and 10.74 mg for males (HR males: 150.08 mg ± 21.14; C males: 139.35 mg ± 18.60) (Table 1.2, Figures 1.3A and 1.3B). Only 7 of the 22 measurements reported a statistically significant ($P < 0.05$) difference in heart mass between HR and C

lines (Table 1.2, Figure 1.3C). ANCOVA indicated that the effect size for heart mass tended to decrease across generations ($P_{Generation}$ = 0.0789, Table 1.2, Figures 1.3C and 1.3D), to be lower in females (P_{Sex} = 0.0966), with no sex*generation interaction ($P_{Generation*Sex}$ = 0.9720, Table 1.2), and older mice tending to have lower effect sizes (P_{Age} = 0.0632).

Discussion

Findings from a meta-analysis of body size and heart mass in HR mice

One objective of the present study was to demonstrate the utility of meta-analytic procedures in reviewing and summarizing results from experimental evolution studies. We proposed that a quantitative approach (e.g., here, an ANCOVA of effect sizes), as opposed to a qualitative one (e.g., do studies fall above/below nominal significance of p = 0.05), would allow us to better summarize data from the HR mouse model, and reveal underlying trends that may not be apparent when examining p-values alone. For example, if had we performed a literature review of the HR mouse model focusing on the statistical significance of studies, we would find that only 12 of 46 measurements (4 before the selection limit, 8 after) were statistically significant for body size and only 7 of 46 measurements (none before the selection limit, 7 after) were statistically significant for heart mass (corrected for body mass). Therefore, we may have reasonably concluded that selection for voluntary wheel-running behavior had not resulted in significant changes to either body size or heart mass. However, by

using a meta-analytic approach, we were able to demonstrate that: (1) effect sizes for body mass and relative heart mass decreased and increased, respectively, before HR mice reached a selection limit for voluntary wheel-running behavior; (2) a plateau in effect sizes for both traits coincides with the generational range during which the selection limits were reached; (3) overall effect sizes for body size and heart mass in males have not significantly changed since the selection limit was reached; (4) effect sizes for body size (and possibly also heart mass) have gotten smaller (i.e., closer to 0) since the selection limit in females.

Effect sizes, p-values, and statistical power

Effect sizes are generally more useful than p-values for summarizing and reporting the results of multiple studies on the same topic (e.g., see Rosenthal and Rosnow 2008; Halsey et al. 2015; Goh et al. 2016; Halsey 2019). Effect sizes provide information on both the magnitude and direction of an effect, whereas p-values can only inform as to the probability that an effect exists. Consistency in the magnitude and direction of an effect size across multiple studies strengthens one's conclusions. Although this is also somewhat true of p-values, they are known to both (1) consistently demonstrate statistically significant levels when sample sizes are sufficiently large, even if effects are relatively non-existent (e.g., see Bartolucci et al. 2011), and (2) fail to reach significant levels when effects are relatively small (Rosenthal and Rosnow 2008;

Sullivan and Feinn 2012; Halsey et al. 2015; Wasserstein and Lazar 2016; Halsey 2019). These phenomena are due to Type I (rejecting the null hypothesis when it is actually true) and Type II errors (accepting the null hypothesis when it is actually false), respectively.

Regarding (1), statistical significance represents the probability that an observed difference between samples from different groups is due to sampling error. Typically, if $p > 0.05$, differences are assumed to be explained by sampling variability. However, larger sample sizes are inherently more likely to resemble the overall population, and very large sample sizes substantially decrease the uncertainty of sampling variability. Therefore, very large samples would yield statistically significant results, even if there were little to no difference between groups (e.g., as seen in Bartolucci et al. 2011). However, this is not an issue for linetype comparisons in the HR mouse model, because d.f. are small and constant (e.g., see the next paragraph). Regarding (2), statistical power (i.e., Type II error rate) represents the sensitivity of a test to detecting an effect when one is present. Power is influenced by both sample size and effect size. When effect sizes are large, the effect of sample size on power is logarithmic, i.e., one can use a relatively small sample sizes and still sufficiently detect existing differences between groups. However, when effect sizes are small, the effect of sample size on power is small and linear, i.e., one must have relatively larger sample sizes to sufficiently detect existing differences between groups. Thus, p-

values are somewhat confounded by their relative dependence on sample sizes, whereas effect sizes are relatively insulated from such effects.

In the HR mouse model, two factors that affect the statistical power for linetype comparisons (i.e., the average values for the 4 HR vs. 4 C lines) are: (a) the number of lines (restricted to 8), which limits power, and (b) the number of mice measured per line, which influences the ability to determine the true line means. Regarding (a), mini-muscle status (described in Section 2.1) is somewhat confounded with linetype, especially in later generations, as described in Castro et al. (2021). Castro et al. (2021) demonstrated that the Type I error rate for mini-muscle effects is close to the expected 5% for $\alpha = 0.05$; however, the Type I error rate for linetype effects was only 1.4%, indicating a reduced capacity to detect linetype differences within the HR mouse model. Regarding (b), increasing amounts of among-line variance decrease statistical power to detect linetype differences by both decreasing the power to detect differences between the average of the 4 HR and the 4 C lines, but also by increasing the overlap between the range of values within HR and C linetypes (akin to extending a folding fan within each treatment group). During earlier generations, this was not much of an issue, as neither random genetic drift nor multiple adaptive responses ("multiple solutions," e.g., see Garland Jr. et al. 2011a; Hannon et al. 2011; Hillis and Garland Jr. 2023) would yet have had much effect.

Another consequence of low statistical power is the overestimation of effect sizes (e.g., as seen in Button et al. 2013). This may explain why effect

sizes in the present meta-analysis are relatively large (e.g., r values of 0.1, 0.3, and 0.5 are traditionally categorized as small, medium, and large effect sizes respectively (Cohen 1988; Lakens 2013)), despite what amounts to modest differences in body size and heart mass (e.g., differences in heart mass after the selection limit between HR and C females is 7.40 mg, which amounts to ~5.34% of average female heart size, Table 1.2). That said, the relative consistency of the observed trends in effect size, as well as Fisher's combined statistical significance being well below nominal significance (e.g., $p < 0.0001$ for the effect sizes of female heart mass after the selection limit), supports the general trends outlined in Section 4.1.

In the present study, effect sizes were calculated from the F-statistics and degrees of freedom (see Section 2.3). However, given that HR and C lines were always compared with 1 and 6 d.f., this resulted in a direct, but non-linear, relationship between r- and p-values (Figure 1.1). This non-linear relationship resulted in two phenomena: (1) effect sizes less than about 0.7 did not result in statistically significant ($p < 0.05$) differences and (2) larger effect sizes did not result in proportionally smaller p-values.

Conclusions and future directions

Prior studies have indicated that neither exhausted additive genetic variance nor counterposing natural selection related to female reproductive success (Girard et al. 2002; Keeney 2011; Careau et al. 2013) are responsible

for the selection limits observed in all four of the selectively bred HR lines of mice. As a complement to the traditional quantitative-genetic explanations for selection limits, a number of studies have sought potential functional limitations either on the ability or willingness (motivation) to engage in voluntary exercise. Our results indicate that, despite a lack of statistical significance in many generations, HR mice have evolved to be smaller and to have larger hearts (relative to body mass) as compared with C mice. Moreover, a plateau in effect sizes for both of these traits coincides with the generational range which the selection for wheel-running behavior was reached.

These correlated responses in body and relative heart mass indicate the presence of genetic correlations with wheel-running behavior, which may have imposed constraints on the evolution of wheel running (e.g., see Weber 1990; Garland Jr. and Carter 1994). To experimentally test this hypothesis, as pointed out in Swallow et al. (1999), selection could be simultaneously applied to both traits (e.g., body mass and wheel running) (e.g., see Wone et al. 2015). With respect to exercise motivation, several lines of evidence have indicated that changes in the reward circuitry of HR mice may underlie a higher motivation for voluntary wheel running (e.g., see Rhodes et al. 2001, 2003, 2005; Rhodes and Garland Jr. 2003; Belke and Garland Jr. 2007; Keeney et al. 2008, 2012; Saul et al. 2017; Thompson et al. 2017). However, studies that would allow a direct comparison of motivation for wheel running across generations, and in relation to the timing of the selection limit, are not available.

Overall, our results demonstrate the utility of applying meta-analytic techniques to long-term selection experiments, particular with regard to how meta-analyses can be used to reveal previously undiscovered trends in existing data. The present study is intended to be part of a broader synthesis of traits related to voluntary exercise, across multiple levels of biological organization, that have been studied in the HR mouse model (e.g., see Khan 2023). Future avenues for study include other characteristics that have been inconsistent with regard to their statistical significance, such as other organs related to exercise (e.g., liver, kidney), skeletal morphology (e.g., see Castro et al. 2021), and the maximal rate of oxygen consumption during forced exercise (VO_2max) (e.g., see Schwartz et al. 2023). Additionally, more information from later generations would allow us to confirm the observed trends in the magnitude of effect sizes for body mass and relative heart mass after the selection limit, particularly with respect to non-breeding females, for which the effect size seems to be decreasing.

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Table 1.1. Summary information from studies used in the meta-analysis. Studies were included if they had body mass and relative heart (ventricle) mass, and followed standard protocol for the HR selection experiment, including: (1) analyses were conducted using SAS Proc Mixed, with linetype as a fixed effect and line nested within linetype as a random effect, (2) all 8 lines were present, (3) there was a non-experimental linetype group. F-statistics and degrees of freedom (always 1 and 6) were used to calculate Pearson's r using: $r = \sqrt{\frac{F}{F+d.f.}}$. A positive effect size indicates that HR mice have a larger value for a given trait than C mice, whereas a negative effect size indicates HR mice have a smaller value for a given trait. Upper and lower 95% confidence limits are provided for each effect size estimate.

Table 1.1. Continued.

					Body mass at dissection					Heart ventricle mass				
Ref	Gen	Sex	AgeAvg	N	Control LSM	HR LSM	HR-C	P	R	Control LSM	HR LSM	HR-C	P	R
LG2 unpublished	1	0	112.33	72	28.95	29.45	0.49	0.4491	0.3138	119.03	120.98	1.95	0.5099	0.2749
LG2 unpublished	1	1	107.93	80	36.20	36.17	-0.02	0.9713	-0.0153	138.37	136.71	-1.66	0.4583	-0.3078
LG3 unpublished	2	0	118.89	79	29.89	29.94	0.05	0.9433	0.0303	125.26	121.35	-3.91	0.0685	-0.6710
LG3 unpublished	2	1	118.89	79	32.25	32.36	0.11	0.8571	0.0765	141.78	140.37	-1.41	0.5731	-0.2364
LG4 unpublished	3	0	115.58	76	32.94	32.55	-0.38	0.5477	-0.2516	118.73	116.72	-2.01	0.3960	-0.3496
LG4 unpublished	3	1	112.43	77	38.60	37.84	-0.76	0.2955	-0.4237	138.29	140.28	1.99	0.3253	0.4007
LG5 unpublished	4	0	114.72	60	32.19	31.90	-0.29	0.6153	-0.2114	117.89	119.11	1.22	0.5426	0.2548
LG5 unpublished	4	1	111.89	72	38.64	38.38	-0.26	0.7540	-0.1327	142.32	144.40	2.08	0.3127	0.4103
LG6 unpublished	5	0	115.23	81	30.14	29.91	-0.23	0.7921	-0.1118	124.69	126.61	1.92	0.4691	0.3008
LG6 unpublished	5	1	112.84	75	36.75	36.50	-0.25	0.7848	-0.1158	137.85	140.65	2.80	0.4023	0.3452
LG7 unpublished	6	0	113.56	72	31.68	31.28	-0.40	0.6992	-0.1633	122.24	125.92	3.69	0.4029	0.3448
LG7 unpublished	6	1	111.36	75	37.15	37.39	0.23	0.7893	0.1133	148.97	154.88	5.91	0.2336	0.4756
LG8 unpublished	7	0	111.59	74	33.25	32.44	-0.81	0.3536	-0.3797	123.66	126.97	3.31	0.3730	0.3657
LG8 unpublished	7	1	107.66	77	36.91	36.69	-0.22	0.8369	-0.0874	155.12	155.56	0.44	0.8720	0.0685
LG11 unpublished	10	0	112.71	76	29.75	29.13	-0.62	0.6200	-0.2086	121.51	123.54	2.03	0.6693	0.1802
LG11 unpublished	10	1	111.35	78	34.25	33.98	-0.26	0.8223	-0.0954	147.47	152.26	4.80	0.2398	0.4701
LG15 unpublished	14	0	115.50	74	31.75	30.64	-1.11	0.2444	-0.4661	131.05	134.41	3.36	0.5226	0.2670
Swallow et. al., 2005	14	0	78.90	81	27.10	24.66	-2.44	0.0313	-0.7523	110.41	112.46	2.05	0.2981	0.4217
LG15 unpublished	14	1	110.29	72	35.19	34.01	-1.18	0.1184	-0.5967	153.87	154.61	0.74	0.9130	0.0465
Swallow et. al., 2005	14	1	78.90	79	34.59	30.83	-3.76	0.0032	-0.8883	132.13	141.25	9.12	0.0606	0.6855
LG19 unpublished	18	0	137.03	65	30.89	28.64	-2.25	0.1543	-0.5539	130.17	139.09	8.92	0.1108	0.6067
LG19 unpublished	18	1	134.62	69	36.98	33.70	-3.27	0.0871	-0.6405	152.28	152.74	0.46	0.8931	0.0571
Garland et al., 2002	22	0	129.50	159	31.35	26.45	-4.90	0.0152	-0.8080	136.40	141.35	4.96	0.3164	0.4075
Garland et al., 2002	22	1	129.50	159	37.26	33.88	-3.39	0.0152	-0.8080	144.48	146.69	2.21	0.3164	0.4075
Gomes et al., 2009	35	0	55.19	242	25.25	23.30	-1.95	0.0498	-0.7073	128.17	138.96	10.79	0.0339	0.7451
Rezende et al., 2006	36	0	94.26	58	29.85	26.20	-3.65	0.0029	-0.8919	125.40	129.50	4.10	0.4294	0.3269
Kelly et al., 2017	37	0	175.60	94	33.11	28.18	-4.93	0.0330	-0.7475	125.89	131.83	5.93	0.2911	0.4272
Kolb et al., 2013	41	1	119.58	68	32.23	29.61	-2.62	0.1222	-0.5918	159.37	172.54	13.18	0.0412	0.7266
Kolb et al., 2010	45	0	0.00	94	27.39	24.56	-2.83	0.0315	-0.7517	133.78	144.44	10.66	0.0379	0.7347
LG50 unpublished	49	0	85.96	96	25.66	23.43	-2.23	0.0662	-0.6751	109.50	120.10	10.60	0.0119	0.8240
Meek et al., 2009	49	0	66.67	49	24.94	22.00	-2.95	0.0405	-0.7282	118.30	126.21	7.91	0.2112	0.4960
Dlugosz Dissertation 2012	51	0	80.58	24	26.29	25.25	-1.04	0.3897	-0.3539	127.09	133.11	6.02	0.2856	0.4316
Dlugosz Dissertation 2012	51	1	80.00	24	34.74	31.46	-3.28	0.1636	-0.5437	141.91	152.79	10.89	0.1077	0.6108
Dlugosz et al., 2013	55	1	97.11	44	34.99	31.38	-3.61	0.1899	-0.5166	147.27	162.93	15.66	0.0334	0.7464
Copes et al., 2015	57	0	110.04	48	28.16	24.72	-3.45	0.1004	-0.6209	118.90	133.38	14.48	0.0214	0.7835
Acosta et al., 2015	59	1	113.00	93	36.85	31.23	-5.62	0.0690	-0.6701	156.46	174.06	17.60	0.0616	0.6836
Singleton et al., 2019	68	1	81.00	90	27.50	25.96	-1.54	0.3918	-0.3525	111.70	122.00	10.30	0.0847	0.6442
Hirmatsu et al., 2017	73	1	81.68	93	30.39	28.15	-2.24	0.1727	-0.5341	119.56	125.23	5.66	0.1348	0.5763
Kay et al., 2019	74	1	61.86	104	39.59	35.04	-4.55	0.0636	-0.6798	121.10	128.60	7.50	0.0799	0.6518
Cadney et al., 2021	76	1	139.69	36	35.41	31.02	-4.39	0.0317	-0.7513	141.30	149.50	8.20	0.2119	0.4954
McNamara Dissertation 2022	81	0	80.93	92	25.30	23.37	-1.93	0.1272	-0.5855	104.83	110.05	5.22	0.1033	0.6169
Schwartz Dissertation Chapter 3 2023	85	0	162.70	84	34.12	31.71	-2.41	0.3711	-0.3671	158.80	162.80	4.00	0.5022	0.2797
Schwartz Dissertation Chapter 3 2023	85	1	135.10	102	38.02	34.30	-3.72	0.0781	-0.6547	166.50	176.80	10.30	0.3575	0.3768
Leszczynski et al., 2023	88	0	137.00	62	34.66	31.07	-3.59	0.1551	-0.5530	152.80	158.30	5.50	0.3961	0.3495
Schwartz et al., 2023	88	0	77.88	50	29.53	27.37	-2.16	0.1565	-0.5515	126.00	129.60	3.60	0.3943	0.3508
New data for body and heart ventricle mass (see Section 2.4 for details)	97	1	88.14	128	32.14	29.89	-2.25	0.0382	-0.7340	128.29	136.36	8.07	0.0599	0.6867

Table 1.2. Measurements from Table 1 were partitioned into four groups by sex (females vs males) and by whether studies occurred before or after the selection limit for voluntary wheel-running behavior (~ generation 30, as per Careau et al. 2013). This yielded 24 values from studies prior to the selection limit (12 for females, 12 for males) and 24 values from studies after the selection limit (13 for females, 11 for males). Two studies were removed as outliers as described in Section 2.3 (both from after the selection limit; 1 female, 1 male). (A) Descriptive statistics (e.g., mean, standard deviation) were calculated using SPSS v28. Values for body mass and relative heart mass are presented separately for High Runner and Control lines, as well as for females and males. The average difference in values between HR and C females (and similarly for HR and C males) are listed such that a positive value indicates HR lines have higher values for a given trait. (B) Fisher's combined probability test (also referred to as Fisher's method) was used to determine an overall p-value for body size and heart mass of each group. The formula used was $\chi^2 = -2\sum_{i=1}^{k}\ln(p_i)$, where χ^2 is the cumulative test statistic, k is the number of studies, and p_i are the p-values from each of the independent studies. This formula yields a chi-squared value, with 2k degrees of freedom, which can subsequently be used to calculate an overall p-value. Statistical significance was evaluated a $P < 0.05$. (C) Effect sizes for body mass and relative heart mass were analyzed using an ANCOVA in SPSS v28, with sex as a fixed effect, and generation and age as covariates. Overall effect sizes of each trait are presented as Estimated Marginal Means (EMM) with associated 95% Confidence Limits (LL = Lower Limit and UL = Upper Limit) for before and after the selection limit. The EMM and associated 95% CL for females and males are also presented. (D) Partial eta-squared (and estimate of effect size for ANOVAs), F-statistics, and p-values from ANCOVAs are presented for generation, age, and sex. ANCOVAs were initially run with a Generation*Sex interaction, which was subsequently removed if not statistically significant (evaluated at $P < 0.05$). (E) Parameter estimates and associated 95% CL from ANCOVAs are presented for generation, age, sex, and the generation*sex interaction (if present). For body mass, a positive estimate indicates a variable is associated with a smaller effect size (given effect sizes are negative for body mass). For heart mass, a positive estimate indicates a variable is associated with a larger effect size (given effect sizes are positive for heart mass).

Table 1.2. Continued.

A. Descriptive Statistics							
		Before Selection Limit			After Selection Limit		
		Mean	Std. Dev.	HR-C	Mean	Std. Dev.	HR-C
Body mass	High Runner Females	29.75	2.36	-1.08	26.56	3.74	-2.76
	High Runner Males	35.15	2.35	-1.09	30.74	2.54	-3.41
	Control Females	30.82	1.76		29.32	4.11	
	Control Males	36.23	1.86		34.15	3.47	
Heart mass	High Runner Females	125.71	8.78	2.29	134.86	14.83	7.40
	High Runner Males	146.70	6.93	2.29	150.08	21.14	10.74
	Control Females	123.42	6.90		127.46	15.57	
	Control Males	144.41	7.23		139.35	18.60	
B. Fisher's Combined Probability Method (p-values)							
		Before Selection Limit			After Selection Limit		
		X-squared	d.f.	p-value	X-squared	d.f.	p-value
Body mass	Female	29.96	24	0.0333	63.28	24	0.0000
	Male	34.08	24	0.0175	45.93	20	0.0003
Heart mass	Female	26.31	24	0.0495	49.27	24	0.0005
	Male	23.42	24	0.0584	48.00	20	0.0001
C. Effect Size Estimated Marginal Means							
		Before Selection Limit			After Selection Limit		
		EMM	95% LL	95%UL	EMM	95% LL	95% UL
Body mass	Overall	-0.299	-0.370	-0.228	-0.618	-0.676	-0.559
	Female	-0.305	-0.405	-0.204	-0.618	-0.698	-0.538
	Male	-0.293	-0.394	-0.193	-0.618	-0.705	-0.531
Heart mass	Overall	0.218	0.092	0.344	0.576	0.511	0.641
	Female	0.209	0.030	0.387	0.520	0.431	0.609
	Male	0.227	0.048	0.405	0.632	0.535	0.730
D. Tests of Between-Subject Effects							
		Before Selection Limit			After Selection Limit		
		ηp2	F	P	ηp2	F	P
Body mass	Generation	0.7765	69.49	0.0000	0.0274	0.54	0.4733
	Age	0.2341	6.11	0.0225	0.0045	0.09	0.7716
	Sex	0.0013	0.03	0.8718	0.1924	4.53	0.0467
	Generation*Sex	P = 0.8963, removed from model			0.2053	4.91	0.0391
Heart mass	Generation	0.2587	6.98	0.0156	0.1617	3.47	0.0789
	Age	0.0931	2.05	0.1674	0.1789	3.92	0.0632
	Sex	0.0011	0.02	0.8833	0.1459	3.07	0.0966
	Generation*Sex	P = 0.4290, removed from model			P = 0.9720, removed from model		
E. Parameter Estimates							
		Before Selection Limit			After Selection Limit		
		Estimate	95% LL	95% UL	Estimate	95% LL	95% UL
Body mass	Generation	-0.045	-0.056	-0.034	-0.002	-0.007	0.003
	Age	0.007	0.001	0.012	0.000	-0.002	0.002
	Female	-0.011	-0.153	0.131	-0.404	-0.801	-0.007
	Male	Set to 0, redundant			Set to 0, redundant		
	Female * Generation				0.006	0.000	0.012
	Male * Generation				Set to 0, redundant		
Heart mass	Generation	0.025	0.005	0.045	-0.003	-0.007	0.000
	Age	-0.007	-0.017	0.003	-0.002	-0.004	0.000
	Female	-0.018	-0.271	0.235	-0.112	-0.246	0.022
	Male	Set to 0, redundant			Set to 0, redundant		
	Female * Generation						
	Male * Generation						

Figure 1.1. Statistical significance vs absolute effect size (calculated using $r = \sqrt{\frac{F}{F+d.f.}}$) for each of the measurements used in the present meta-analysis. As described in Section 2.3, degrees of freedom for each study were always 1 and 6. Therefore, r and the original p-values are directly, but not linearly, related. Dashed line is set to P = 0.05. Note that this non-linear relationship results in (1) effect sizes less than about 0.7 not yielding statistically significant differences and (2) larger effect sizes not yielding proportionally smaller p-values.

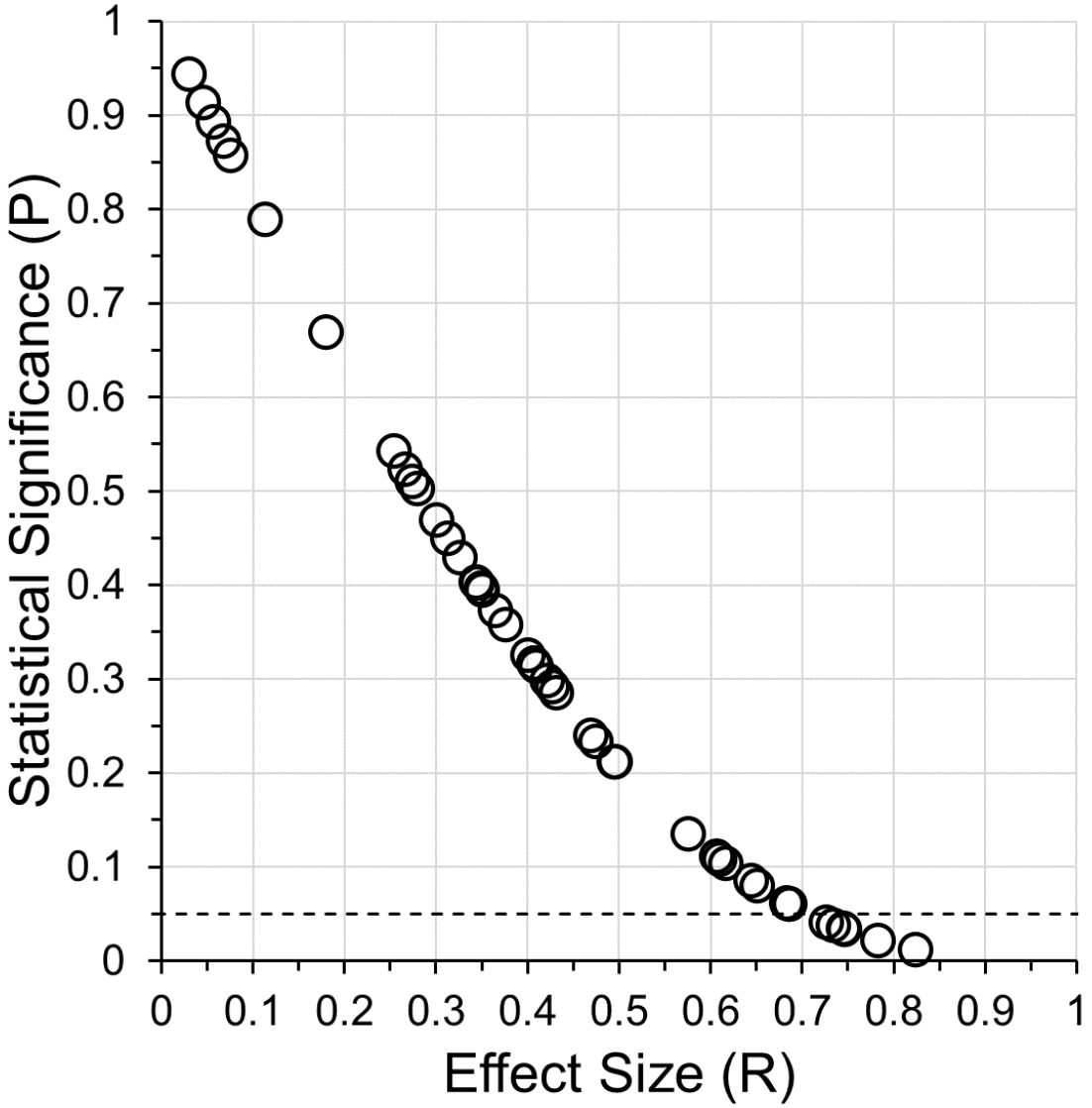


Figure 1.2. Values for body mass. (A) Least Squares Means (LSMs) (from analyses in SAS Proc MIXED) for body mass from the individual studies used in the present meta-analysis. High Runner lines are in red and Control lines are in blue. Females are denoted by circles, males by squares. Open shapes indicate studies that occurred before the selection limit for voluntary wheel-running behavior (~generation 30), whereas closed shapes indicate studies that occurred after. (B) Differences in the LSMs for body mass between HR and C lines. A positive value indicates HR lines have higher values for a given trait. (C) Original p-values from individual studies used in the present meta-analysis. Dashed line is set to P = 0.05. Note that few are < 0.05. (D) Effect sizes from individual studies, calculated from the F-statistics and degrees of freedom (always 1 and 6), using $r = \sqrt{\frac{F}{F+d.f.}}$. A negative effect size indicates that HR lines have a smaller value for a given trait. Effect sizes in the present study may be relatively large, despite somewhat small differences in body mass (e.g., differences in body mass for females after the selection limit is 2.76 g, which amounts to ~4.82% of average female body mass, Table 2), due to relatively low statistical power (as outlined in Section 4.2). That said, the relative consistency of the observed trends in effect sizes below strengthens the conclusions drawn in Section 4.1.

Figure 1.2. Continued.

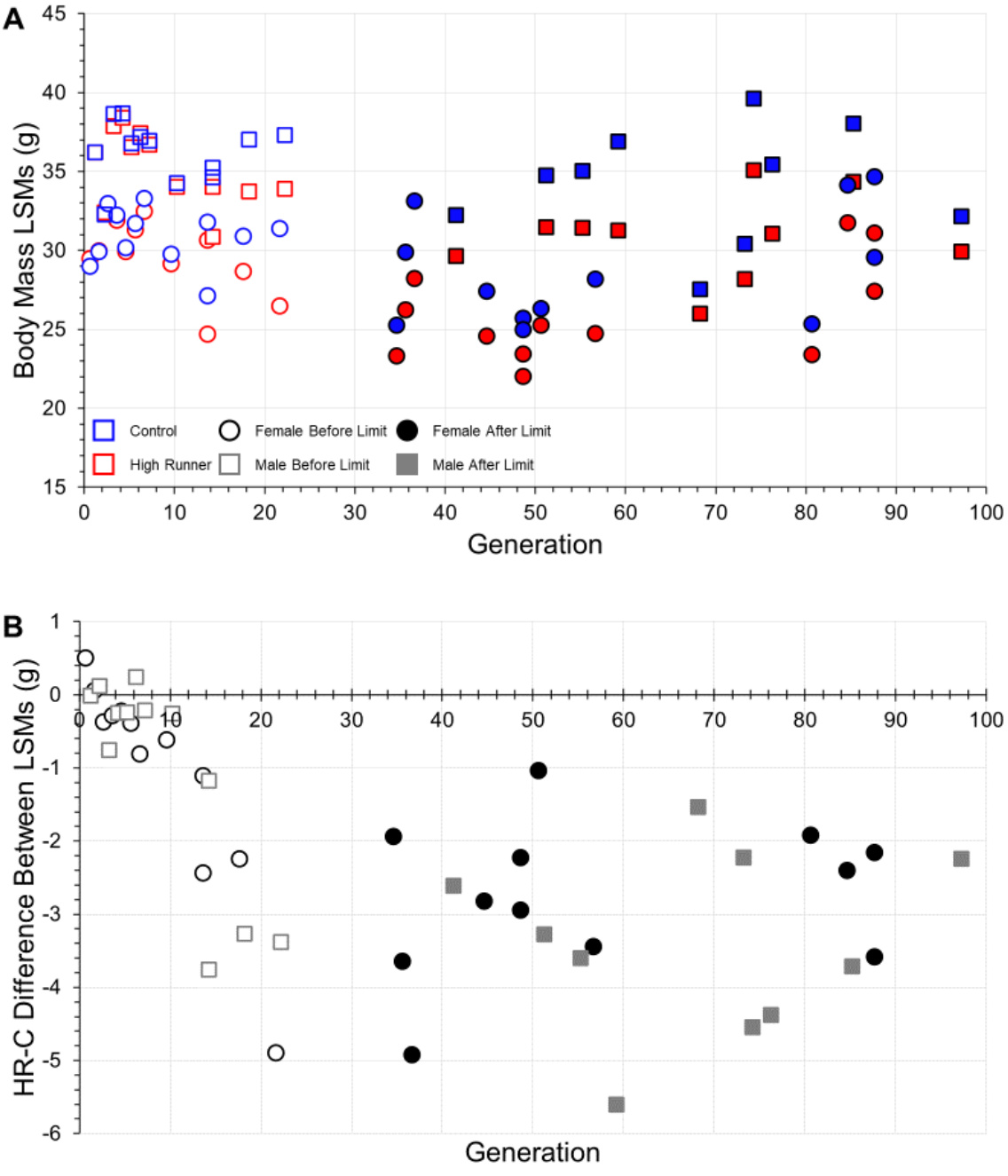


Figure 1.2. Continued.

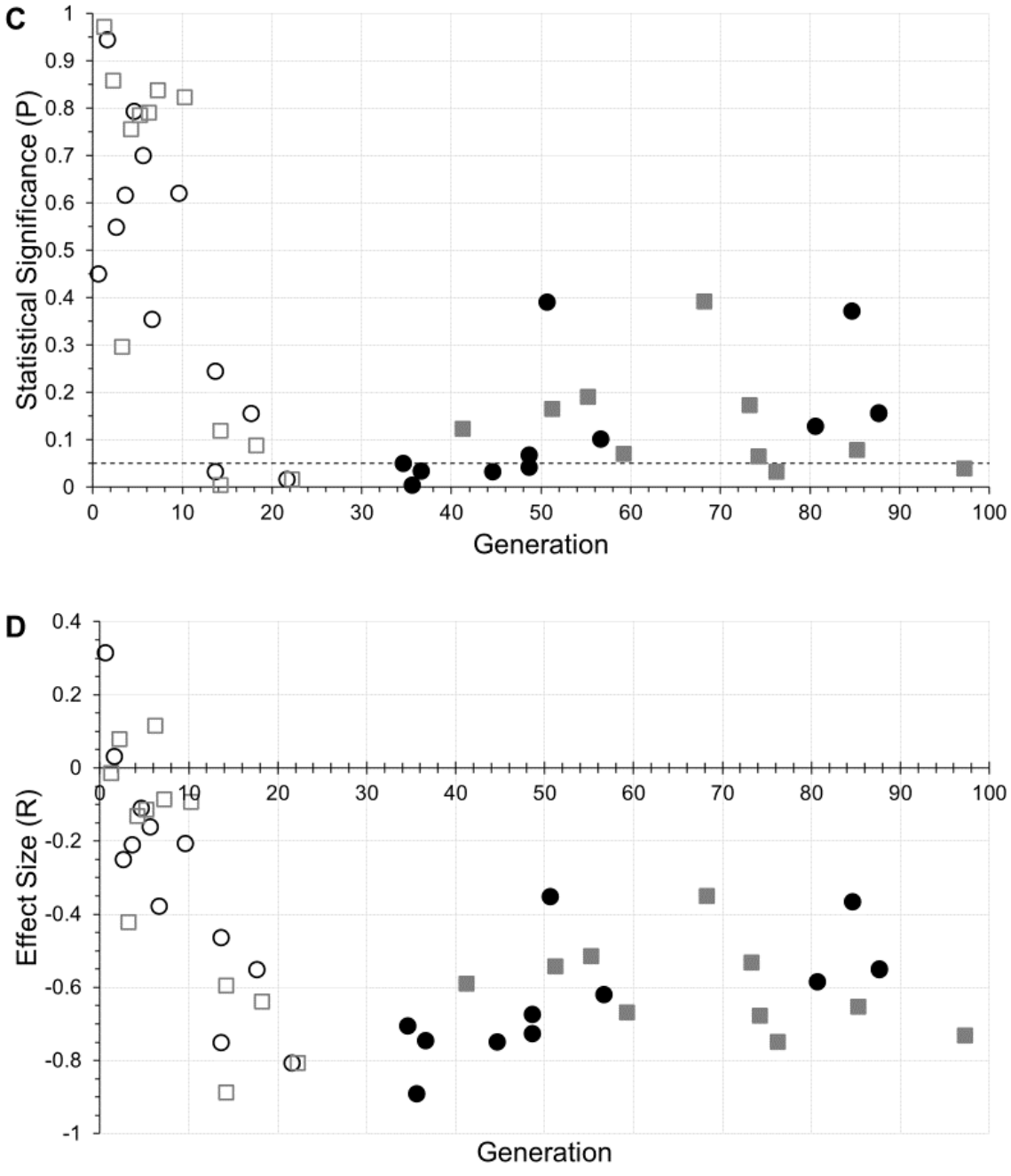


Figure 1.3. Values for relative heart mass. (A) Least Squares Means (LSMs) (from analyses in SAS Proc MIXED) for relative heart mass from the individual studies used in the present meta-analysis. High Runner lines are in red and Control lines are in blue. Females are denoted by circles, males by squares. Open shapes indicate studies occurred before the selection limit for voluntary wheel-running behavior, whereas closed shapes indicate studies occurred after. (B) Differences in the LSMs for relative heart mass between HR and C lines. A positive value indicates HR lines have higher values for a given trait. (C) Original p-values from individual studies used in the present meta-analysis. Dashed line is set to P = 0.05. Note that few are < 0.05. (D) Effect sizes from individual studies, calculated from the F-statistics and degrees of freedom (always 1 and 6), using $r = \sqrt{\frac{F}{F+d.f.}}$. A negative effect size indicates that HR lines have a smaller value for a given trait. Effect sizes in the present study may be relatively large, despite somewhat small differences in body mass (e.g., differences in body mass for females after the selection limit is 7.40 mg, which amounts to ~5.34% of average female heart mass, Table 2), due to relatively low statistical power (as outlined in Section 4.2). That said, the relative consistency of the observed trends in effect sizes below strengthens the conclusions drawn in Section 4.1.

Figure 1.3. Continued.

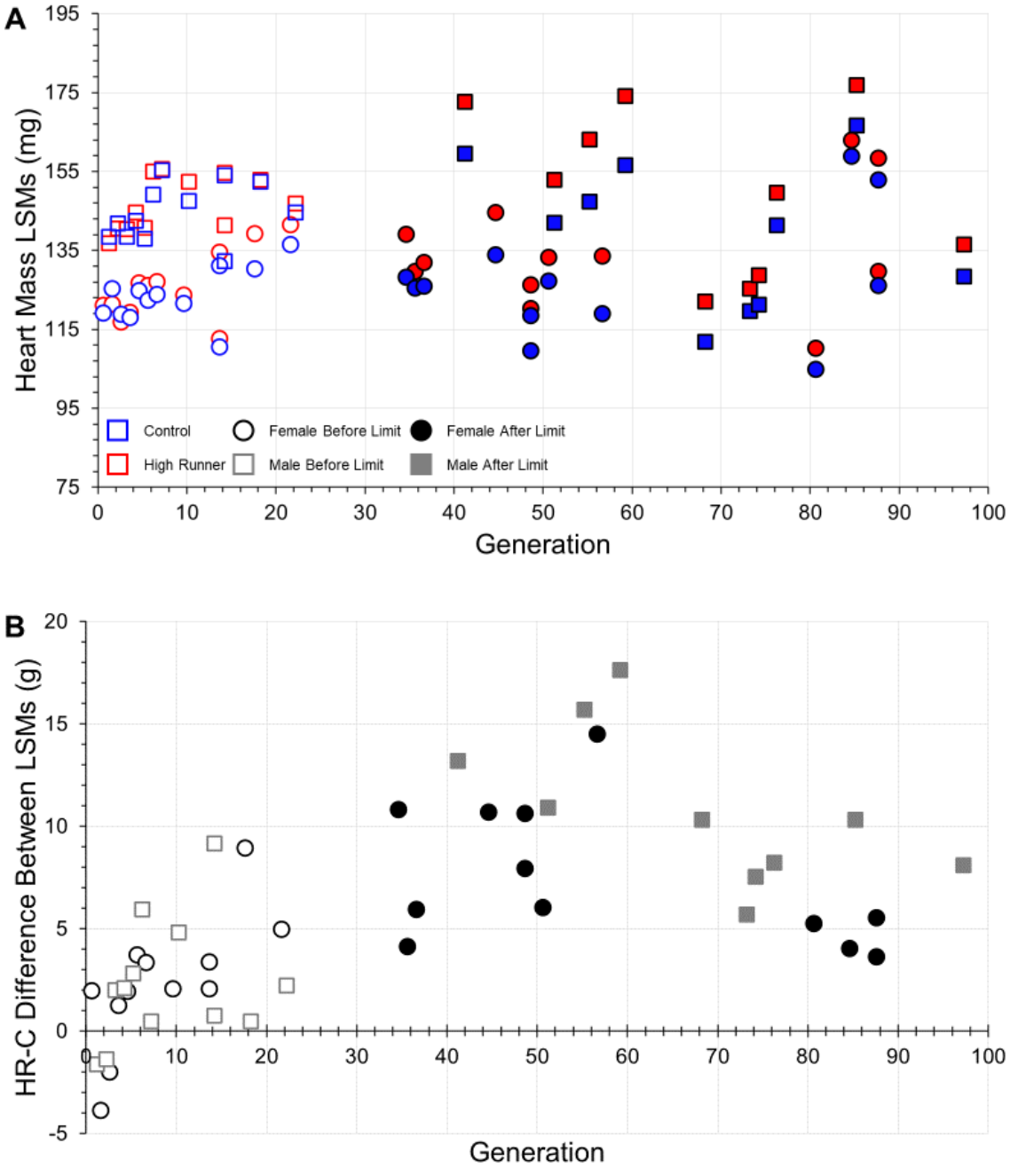
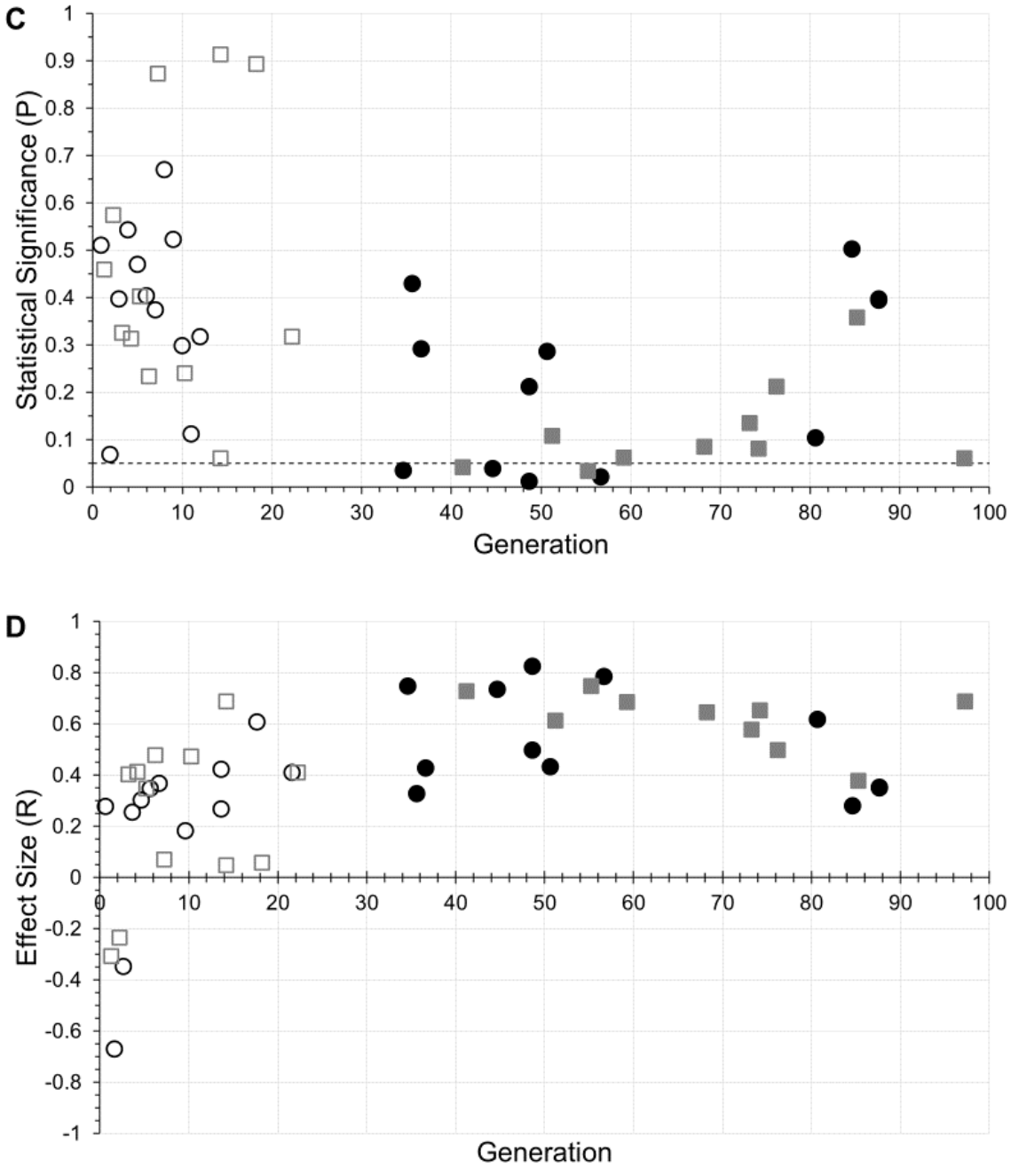


Figure 1.3. Continued.



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

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.

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 Jr. 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 Jr. 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 Jr. 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 Jr. 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 Jr. 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 Jr. 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 Jr. 2003; Wallace and Garland Jr. 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).

Methods

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 Jr. 2003; Careau et al. 2013; Wallace and Garland Jr. 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 Jr. 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 Jr. 2001; Hiramatsu and Garland Jr. 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).

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 (e.g., Rezende et al. 2005, 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.

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.

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.

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.

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 2.1, 2.2). 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 Jr. 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

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 2.1A, Table 2.4). This increase in wheel-running behavior was caused primarily by a significant increase in average running speed (+142%: Figure 2.1C, Table 2.4), accompanied by a non-significant increase in running duration (+27%: Figure 2.1B, Table 2.4). Additionally, maximum running speed was significantly higher in HR mice (+94%: Figure 2.1D, Table 2.4).

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 2.1 and 2.2). 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 2.1 and 2.2).

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 2nd of the two VO_2 trials was higher ($t = 2.554$, $P = 0.013$). Additionally, after regressing each VO_2 on the corresponding body mass for that individual, 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 2.2A, Table 2.1). 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 2.2C, Table 2.1). Mini-muscle status did not significantly affect either VO_2max or BMR (Figure 2.2A and C, Table 2.1). However, when lean mass was used as a covariate (see above), VO_2max was ~4.6% higher in mini-muscle mice (Figure

2.2B, Table 2.1). Mini-muscle mice were also significantly more exhausted after their VO_2max trials (Table 2.1).

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 2.3, Table 2.2). Mini-muscle mice had ~50% less hindlimb muscle mass, as expected (Garland Jr. et al. 2002; Houle-Leroy et al. 2003), had significantly larger livers and lungs, and tended to have larger kidneys and spleens (Figure 2.3, Table 2.2).

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 2.3). However, residual VO_2max and ventricle mass were significantly positively correlated among all individuals and for line means (Table 2.3). BMR and ventricle mass were significantly and positively correlated among line means (i.e., 4 replicate HR and 4 replicate C LSMs), but not among individuals (Table 2.3). Mass-corrected VO_2max and BMR were not significantly correlated with any other lower-level trait (Table 2.3). 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 Table 2.5.

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 Jr. 2003; Wallace and Garland Jr. 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 Jr. 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 data was 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 2.1, Figure 2.2A), but did not have significantly higher BMR (+6.5%, Table 2.1, Figure 2.2C). Additionally, VO_2max and BMR were not correlated at any level (e.g., among individuals or replicate lines, Table 2.3). 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 2.3), and between ventricle mass and BMR among replicate lines (but not among individuals; Table

2.3), 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; Książek et al. 2004, Gębczyński and Konarzewski 2009, Sadowksa et al. 2015, Wone et al. 2015).

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 Jr. 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 2.4, Figure 2.1), without large modifications to underlying morphological or physiological traits (here, only a 13.6% increase in VO_2max (Table 2.1, Figure 2.2A), a 6.5% increase in BMR (Table 2.1, Figure 2.2C), and no statistically detectable changes in organ masses or hematocrit (Table 2.2, Figure 2.3).

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Table 2.1. VO_2max, BMR, and associated whole-body, lean, and fat mass. 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 log10 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.

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)

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

Table 2.2. Organ masses and associated whole-body, lean, and fat mass. 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.

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

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

Table 2.3. Correlations at the level of individual variation. 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 VO_2max 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 VO_2max and BMR in relation to lower-level traits.

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

Table 2.4. Female sibling wheel running data. Statistical analyses corresponding to Figure 1. Least Squares Means (LSM), Standard Errors (SE), F-statistic, and P-values are from SAS Procedure Mixed analyses with linetype as a fixed effect and line nested within linetype as a random effect. Body mass was analyzed with age as a covariate. Wheel-running behavior and its components, running speed and duration, were analyzed with age and wheel freeness as covariates.

Trait	N	HR/C	High Runner		Control		Linetype		
			LSM	SE	LSM	SE	d.f.	F	P
Body mass before wheel access (g)	372	0.912	22.33	0.940	24.47	0.604	1,6	3.68	0.1034
Wheel-running distance (revs/day)	372	3.013	12,582	302.1	4,176	280.1	1,6	415.82	<.0001
Wheel-running duration (min)	372	1.266	576.9	37.80	455.7	32.12	1,6	5.97	0.0502
Average running speed (revs/min)	372	2.424	21.75	1.082	8.97	0.2670	1,6	131.24	<.0001
Maximum running speed (revs/min)	370	1.943	35.86	1.266	18.46	0.5031	1,6	163.13	<.0001

Table 2.5. Correlations at the level of individual variation (above diagonal) and line means (below diagonal). Above the diagonal: 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 lower-level traits. Below the diagonal: 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 lower-level traits. Asterisks indicate P < 0.05, unadjusted for multiple comparisons.

Individual Mice (above diagonal)									
Trait		Ventricle	Muscle	Hematocrit	Lung	Brain	Liver	Kidney	Spleen
Ventricle	r	---	0.165	0.134	0.088	0.158	0.236*	0.134	0.047
	P	---	0.165	0.275	0.467	0.190	0.046	0.262	0.696
	N	---	72	68	70	70	72	72	72
Muscle	r	-0.050	---	0.027	0.135	0.224	0.141	-0.231	-0.056
	P	0.899	---	0.827	0.267	0.063	0.236	0.050	0.643
	N	9	---	68	70	70	72	72	72
Hematocrit	r	0.203	0.425	---	0.378*	-0.171	-0.166	-0.177	-0.099
	P	0.600	0.254	---	0.002	0.171	0.177	0.148	0.420
	N	9	9	---	66	66	68	68	68
Lung	r	-0.117	-0.420	-0.608	---	-0.002	0.009	-0.188	0.076
	P	0.765	0.260	0.083	---	0.985	0.940	0.119	0.534
	N	9	9	9	---	69	70	70	70
Brain	r	0.373	-0.571	-0.428	0.285	---	0.338*	0.099	0.376*
	P	0.323	0.109	0.250	0.457	---	0.004	0.415	0.001
	N	9	9	9	9	---	70	70	70
Liver	r	-0.283	-0.588	-0.313	0.432	0.168	---	0.230	0.345*
	P	0.461	0.096	0.412	0.245	0.665	---	0.052	0.003
	N	9	9	9	9	9	---	72	72
Kidney	r	0.084	-0.646	-0.681*	0.414	0.777*	-0.003	---	0.069
	P	0.829	0.060	0.043	0.268	0.014	0.994	---	0.564
	N	9	9	9	9	9	9	---	72
Spleen	r	-0.403	-0.614	-0.351	0.337	0.403	0.797*	0.294	---
	P	0.282	0.078	0.354	0.375	0.282	0.010	0.443	---
	N	9	9	9	9	9	9	9	---
Line Means (below diagonal)									

Figure 2.1. 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 2.1. Continued.

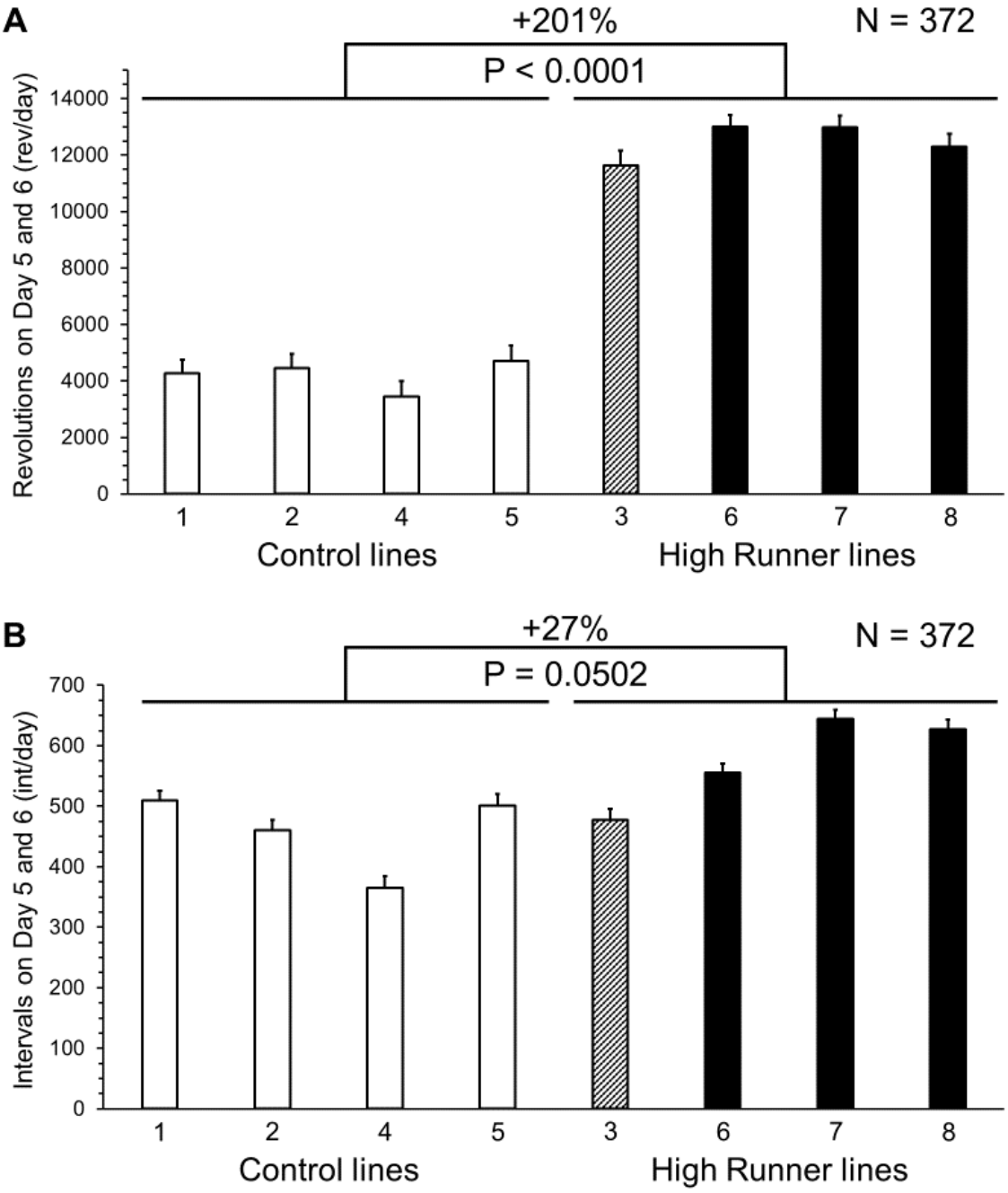


Figure 2.1. Continued.

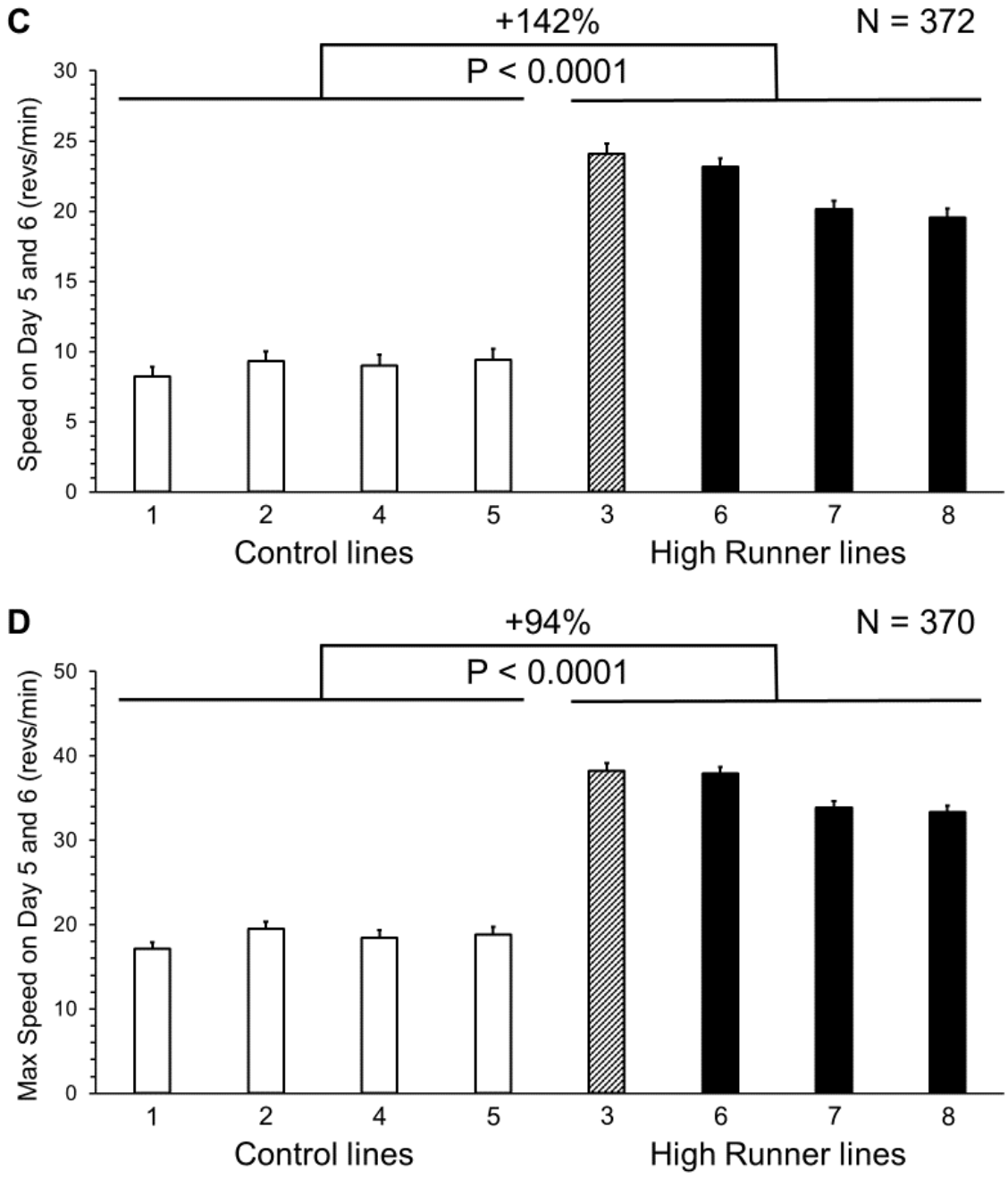


Figure 2.2. Scatterplot of VO_2max and BMR versus body mass or lean mass. P-values are significance levels from models in SAS Procedure Mixed with linetype and mini-muscle status as fixed effects, replicate line as a random effect nested within linetype, and body (either whole-body or lean) mass and age as covariates.

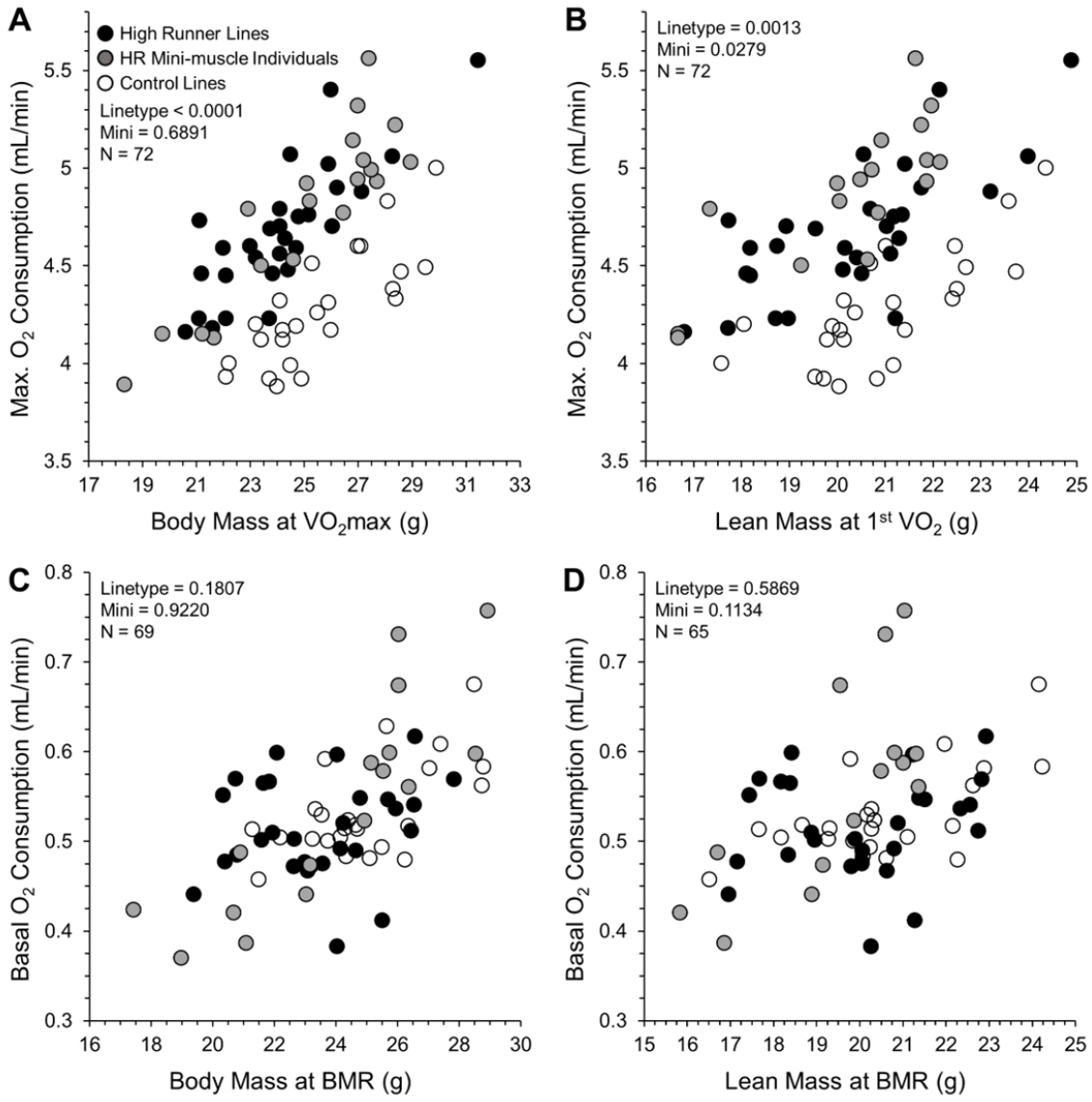
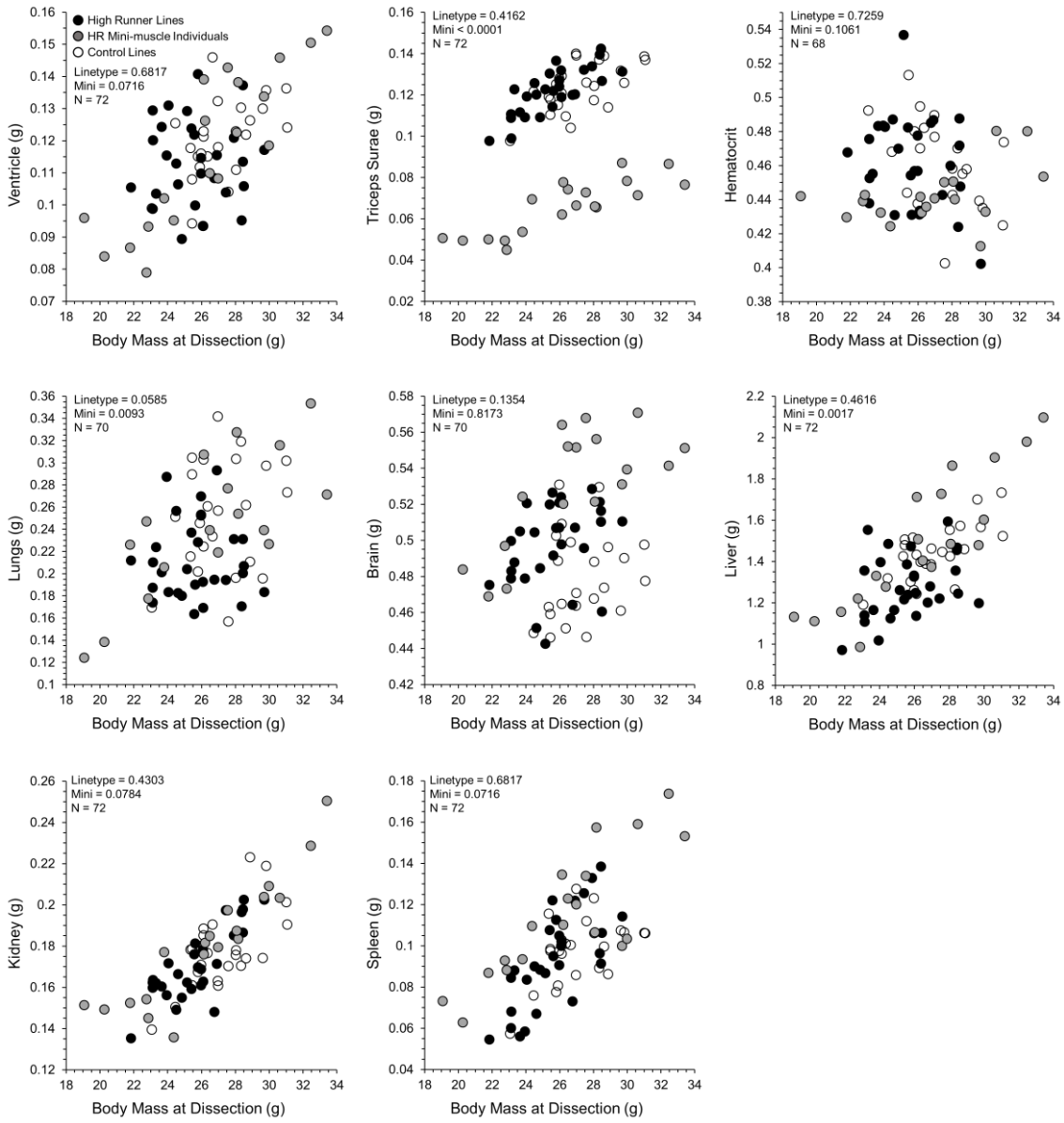


Figure 2.3. Organ masses in relation to body mass. Values are significance levels from models in SAS Procedure Mixed with linetype and mini-muscle status as fixed effects, replicate line as a random effect nested within linetype, and body mass (except for hematocrit) and age as covariates.



3. Maternal exercise before, during, and after pregnancy has minimal effects on offspring physical activity or body composition in mice

Abstract

The prevalence of obesity has substantially increased over the past 60 years, especially in Western societies. However, energy balance (and thus body composition) is complex, with multiple factors likely exacerbating the consequences of excessive caloric intake and/or insufficient physical activity. For example, adult body composition may be "programmed" by developmental experiences, where changes during key periods of growth and development can have long-lasting effects on adult phenotype. In humans, mothers with high gestational weight gain give birth to heavier offspring, who are then more likely to be obese as adults, leading some to suggest an altered maternal energetic state could be an important factor in the development of offspring obesity. Although moderate exercise during pregnancy has clear beneficial health outcomes for mothers (e.g., improved post-partum recovery), benefits to offspring health have been much less clear.

Here, we used a novel mouse model to test for the effects of maternal exercise before, during, and after pregnancy on offspring physical activity and body composition. Mice were from generations 84, 85, and 86 of an ongoing artificial selection experiment for high voluntary wheel-running behavior. Briefly, at ~6-8 weeks of age mice are housed with access to an exercise wheel (1.12-m

circumference) for 6 days. For four replicate High Runner (HR) lines, the highest-running male and female from within each of 10 families are chosen as breeders for the next generation (with no sibling pairs). For four replicate Control (C) lines, breeders are also chosen within family, but without regard to wheel running. In the present study, at 12 weeks of age, 80 prospective dams (40 HR, 40 C) were housed individually with wheels and 97 others were housed without wheels. Dams had continued wheel access two weeks prior to breeding, 18 days with males (i.e., during breeding), during gestation, and up until 10 days postpartum, after which they were housed without wheels until weaning (21 days postpartum). Throughout this period, dam behavior was observed 3 times per day. Offspring were measured for whole-body, lean, and fat mass at several points during their ontogeny. Offspring spontaneous physical activity, voluntary exercise (wheel running), and food consumption were also measured. Finally, grand-offspring wheel running, food consumption, and body composition were measured.

Females from the HR lines ran more revolutions/day (mainly by running faster) than those from C lines when individually housed and in the days leading up to parturition, but not after parturition. Nursing accounted for most maternal behavior in all experimental groups. During post-partum days 1-5, HR and C dams with wheels tended to exhibit less maternal behavior than those without wheels (wheel access $p = 0.0672$). During post-partum days 6-10, HR dams with wheels continued to exhibit less maternal behavior than those without wheels,

whereas C dams with wheels exhibited more maternal behavior than those without (linetype X wheel access interaction $p = 0.0218$). During post-partum days 11-20 (when no dams had wheels), there were no significant differences in activity or maternal behaviors. Birth success (i.e., did a dam successfully give birth) did not differ among groups, but C dams with wheels were less likely to have litter death between birth and weaning than those without wheels, whereas the opposite was true for HR dams (linetype X wheel access interaction $p = 0.0447$). Additionally, the sex ratio (proportion of females to litter size) at weaning was significantly higher in dams with wheels for both HR and C lines, due primarily to a decrease in the number of male offspring. Offspring from dams with wheels had delayed eye opening, and were smaller at weaning and sexual maturity (but at no other time) due to a slight decrease in lean, but not fat, mass. Despite these developmental delays, maternal wheel access had no significant effects on offspring food consumption, spontaneous or voluntary physical activity when measured as adults. Furthermore, maternal wheel access did not affect reproductive characteristics of offspring, nor their adult body composition or relative organ masses. Finally, grand-offspring were unaffected for physical activity, food consumption, body size or composition, or reproductive traits. Overall, our results indicate that, at least for these lines of mice, maternal exercise does not have long-lasting impacts on offspring spontaneous physical activity, voluntary physical activity, or body composition.

Introduction

Obesity (i.e., excessive accumulation of body fat) is the cumulative result of a chronic imbalance between energy intake and expenditure, with many citing excessive caloric intake and insufficient physical activity as primary causes (Miller et al. 1997; Tremblay and Willms 2003; Prentice and Jebb 2008). Although the exact energy imbalance needed to accumulate excess body fat varies, it is consistently small (Plachta-Danielzik et al. 2008; Schoeller 2009; Mabire 2016); therefore, small changes to energy intake or expenditure should be effective in obesity-management strategies. However, interventions related to diet and exercise often fail to demonstrate long-term success, despite several decades of public health efforts (Swift et al. 2013; Rowland 2016). Energy balance is complex, with other factors likely exacerbating the consequences of excessive caloric intake and/or insufficient physical activity.

Early-life experiences, especially during critical periods of development and growth, can have long-lasting effects on adult health (Barker 2007; Hanson and Gluckman 2008; Garland Jr. et al. 2017), and various studies demonstrate that adult body composition can be partially “programmed” by such developmental experiences. In human beings, for example, mothers who experience high gestational weight gain tend to give birth to heavier offspring (Vohr and McGarvey 1997; Hammami et al. 2001; Haugen et al. 2014), who are then at higher risk of obesity (Yu et al. 2011; Mamun et al. 2014; Chiavaroli et al. 2016; Godfrey et al. 2017) as children, adolescents, and adults. Additionally,

these offspring are at higher risk of other comorbidities, such as cardiovascular disease (Curhan et al. 1996b,a; Allcock et al. 2009; Skilton et al. 2014; Gaillard 2015) and Type II diabetes (Wei et al. 2003; Johnsson et al. 2014). For example, in a study using birth records from 37,709 participants, high maternal BMI (>30) at the first antenatal visit (7-12 weeks into pregnancy) was associated with an increase in all-cause mortality of adult offspring (hazard ratio of 1.35), and an increased risk of hospital admission for a cardiovascular event (hazard ratio of 1.29), when compared with those whose mothers had a normal BMI (18.5-24.9) (Reynolds et al. 2013). Accordingly, weight-management strategies before and during pregnancy have been identified as potential interventions for reducing offspring obesity. However, although the impact of maternal diet has been documented in both human and animal models (Wu et al. 1998; Suzuki 1999; Wu and Suzuki 2006; Agha et al. 2014; Hiramatsu et al. 2017; Montalvo-Martínez et al. 2018), the effect of maternal exercise remains unclear (Agha et al. 2014; Perales et al. 2016).

Maternal exercise during pregnancy has a number of clear, beneficial health outcomes for mothers (e.g., reduced risk of gestational diabetes and improved post-partum recovery (Davies et al. 2003; Pivarnik et al. 2006; Borodulin et al. 2008)), but potential benefits to offspring health -- especially once offspring reach adulthood -- have been much less studied. Indeed, maternal exercise has only recently received attention as a preventative strategy for offspring obesity (Birdsall et al. 2009; Wasenius et al. 2017). Currently, the CDC

(Center for Disease Control, U.S.) and WHO (World Health Organization) recommend ~30 minutes per day (~150 minutes per week) of moderate-intensity aerobic activity (e.g., a brisk walk) for pregnant women (Piercy et al. 2018). However, in a 2018 survey of 9,345 Polish women, only 52% reported having performed some exercise during pregnancy, and 39% of participants were told by others that physical activity during pregnancy was bad for the baby's health (Walasik et al. 2020). Concerns regarding potential negative effects of exercise during pregnancy still remain and may be deterring women from exercising during critical periods of offspring development. Given the difficulty of experimental studies during human pregnancies, pre-clinical animal models are important to consider.

Here, we investigated the effects of maternal exercise before, during, and after pregnancy on offspring physical activity and body composition using mice from a long-term artificial selection experiment for high voluntary wheel-running behavior. Briefly, the High Runner (HR) lines of mice have been bred for voluntary activity on wheels, an energetically demanding behavior that is likely to influence numerous aspects of their biology (Koteja et al. 1999; Swallow et al. 2001; Rezende et al. 2009; Copes et al. 2015). As expected, many adult traits related to exercise ability and/or motivation have been shown to differ between the four replicate HR lines and non-selected Control (C) lines, such as home-cage activity (spontaneous physical activity) when housed without access to wheels (Malisch et al. 2009), maximum oxygen consumption (VO_2max) (Rezende

et al. 2005; Kolb et al. 2010; Schwartz et al. 2023), brain size (Kolb et al. 2013), and relative fat mass (Girard et al. 2007; Hiramatsu and Garland Jr. 2018). Yet, whether HR females are more physically active than C females when gestating or nursing pups is unknown. But given that HR mice appear to have a stronger motivation to engage in physical activity (e.g., see Rhodes et al. 2005; Belke and Garland Jr. 2007; Saul et al. 2017 p. 20; Kay et al. 2022), any effects of maternal exercise on offspring should be more apparent in HR mice.

Previous studies of maternal behaviors have reported few, if any, significant differences between HR and C lines (Girard et al. 2002; Keeney 2011; Hiramatsu et al. 2017). However, in these studies, mice were housed without wheels. We hypothesized that HR dams may behave and allocate resources differently when faced with an additional energetic challenge, such as from voluntary wheel running. With a wheel available, mice would face a choice between competing "drives" (e.g., Stults-Kolehmainen 2023), i.e., voluntary exercise versus maternal care. To the extent that wheel running was engaged, the energy and/or time available for rearing pups might be reduced. Thus, we expected that any deficits in maternal behavior due to wheel running (if present) would be exacerbated in HR dams as compared with C dams.

We also hypothesized that exercise during pregnancy and/or after birth could improve indicators of offspring health, such as body composition (less fat, more lean mass), exercise behavior, or both. We expected that the differences in wheel running typically observed between HR and C lines would likely

continue during pregnancy and after birth (albeit, to a lesser degree), due to the HR lines having higher motivation for running. However, the higher levels of physical activity normally exhibited by HR lines may be "excessive" during pregnancy and/or after birth, even if this were partly compensated by a reduction in other aspects of physical activity (e.g., see Garland Jr. et al. 2011b; Copes et al. 2018). Therefore, we also hypothesized that the higher physical activity of HR lines (if present) might result in detrimental effects on offspring growth and development.

We conducted this experiment using three generations: (1) the maternal generation, wherein we measured exercise behavior and maternal care in the presence and absence of a running wheel; (2) the offspring generation, wherein we measured body composition at several points in their ontogeny, tested for changes in behaviors related to physical activity, and measured relative organ masses; and (3) the grand-offspring generation, wherein we again measured body composition and physical activity. Figure 3.1 presents an experimental timeline for (A) the maternal generation, (B) the offspring generation, and (C) the grand-offspring generation.

Methods

Mouse model

Mice for this experiment were from generations 84 (maternal), 85 (offspring), and 86 (grand-offspring) of a long-term artificial selection experiment

for high voluntary wheel-running behavior (Swallow et al. 1998; Garland Jr. 2003; Careau et al. 2013; Wallace and Garland Jr. 2016). Briefly, the founder population consisted of 224 outbred Hsd:ICR strain (Hauschka and Mirand 1973) house mice (*Mus domesticus*). Four lines were designated as Control (C) lines and four were designated as High Runner (HR) lines. In each subsequent generation, mice have been housed four per cage from weaning (21 days of age) until sexual maturity (~6-8 weeks of age), at which time they are housed individually with access to an exercise wheel (1.12-m circumference) for six days. Mice are weighed at the start and end of this wheel-access period, during which wheel revolutions are recorded in one-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 (within-family selection; no sibling pairings allowed). For the four replicate C lines, breeders are chosen without regard to wheel running. Once breeding pairs have been chosen, males and females are housed together for 18 days, with water and food (Harlan Teklad Laboratory Rodent Diet [W]-8604) available *ad libitum*. After 18 days, males are removed from cages and pregnant dams are given a breeder diet (Harlan Teklad Laboratory Breeder Diet [S-2335]-7004) until offspring are weaned at 21 days of age.

The original base population of mice used to start the selection experiment included individuals with hindlimb muscles that are ~50% smaller than normal-muscle individuals (Garland Jr. et al. 2002; Houle-Leroy et al. 2003). This “mini-

muscle" phenotype is caused by an autosomal, Mendelian recessive, single-nucleotide polymorphism that 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, and eventually disappeared from the C line, became fixed in HR line 3, and remains polymorphic in HR line 6 (Kelly et al. 2013). In this experiment, mini-muscle status was accounted for when known, as described in section 2.10.

Maternal wheel-running behavior

Breeding pairs were established using the protocols described above. The pool of available breeders was then partitioned into two groups: those that would retain access to a wheel until 10 days after offspring were born, and those that would not. At ~12 weeks of age, 80 prospective dams (40 HR, 40 C) were weighed and individually housed with access to wheels. All other prospective dams were housed without access to wheels. Additional breeding pairs were assigned to the "no wheels" cohort as a precaution to ensure adequate genetic diversity for the ongoing artificial selection experiment. For dams with wheels, running (females only) was recorded for two weeks prior to pairing breeders. Males were then added to cages at 14 weeks of age, and wheel-running activity was continuously recorded. Wheel running recorded during this period does not solely reflect running of dams, as it was not possible to determine whether the male, female or both were running at any given moment. After 18 days, males

were removed (in accordance with standard procedure). Dams within the wheel access group were given continued wheel access until 10 days postpartum to determine whether voluntary exercise would alter maternal behavior (e.g., nursing), after which they were weighed and housed with their pups, without wheels, until weaning (21 days postpartum). No dam housed its pups within the wheel, nor were offspring observed to move within running wheels. Some prospective dams died, several failed to given birth, and others failed to rear a successful litter to weaning (21 days postpartum); all of these were excluded from relevant analyses (see Table 3.4 for details).

Maternal behavioral observations

From offspring birth until weaning, we observed and recorded the behavior of dams three times daily at equally spaced intervals coinciding with 1-hour prior to lights being turned on (at 7:00 h), mid-day, and 1-hour after lights off (at 19:00 h; under red light), as previous studies using this mouse model have shown increased activity at the beginning and end of the lights-off period, and decreased activity at mid-day (Malisch et al. 2008, 2009). The instantaneous behavior of each dam, as well as any of three offspring developmental markers (eyes open, pup locomotion, solid food consumption), was noted (allowing up to 5 seconds of observation time to ensure accuracy). For analysis, observed behaviors were assigned to one of three pre-determined categories: “maternal” (nursing, resting with, grooming, or carrying pups), “maintenance” (eating,

drinking, or sleeping), and “active” (digging, self-grooming, walking around cage, climbing bars of cage lid, running within wheel, or chewing on bars of cage). Observations were pooled into the following categories for analysis: total observations (3x daily over 20 days, i.e., 60 observations), morning, afternoon, and evening observations (each being 1x daily over 20 days, i.e., 20 observations), days 1-5, 6-10, 11-15, and 16-20 (each being 3x daily over 5 days, i.e., 15 observations). Data are presented and analyzed as a proportion of total observations for each period, i.e., a result of 0.50 would indicate that ½ of all observations in a period (e.g., morning observations) were from a given category (e.g., maternal behavior).

Reproductive success

Breeding pairs were established using the standard protocol for the HR selection experiment (see above) but were also paired by maternal wheel access for the offspring generation (but not grand-offspring), i.e., females whose mothers had wheel access were mated with males whose mothers also had wheel access, and vice versa. From the time breeding pairs were established until offspring were weaned, each cage was inspected at least once per day, and information pertinent for breeding success (e.g., if breeding pairs needed to be separated due to fighting, offspring births and/or deaths) was recorded. This information was used to evaluate key aspects of breeding success for each generation, e.g., the ability to successfully conceive and give birth (birth

success), successfully rear offspring until weaning (wean success), and litter size at weaning.

Whole-body, lean, and fat mass

Mice were weighed several times as part of the routine selection protocol: at weaning (21 days of age), at the start of wheel access (~6-8 weeks of age, i.e., sexual maturity), at the end of wheel access (1 week later), and for female mice, at their offspring's weaning. Prospective dams (maternal generation) were also weighed before and after wheel access (noted above), and offspring were weighed before and after measurement of home-cage activity (noted below) and prior to dissection (noted below). Additionally, body composition of offspring and grand-offspring was measured using non-invasive quantitative magnetic resonance (EchoMRI-100; Echo Medical Systems LLC, Houston, Texas, USA), which independently calculated fat and lean mass (Hiramatsu and Garland Jr. 2018). These measurements were taken at weaning, at the start and end of wheel access, at offspring weaning, and prior to dissection (offspring generation only).

Wheel-running behavior and food consumption

As noted above, mice are routinely weighed and housed individually with access to a wheel for a period of 6 days at ~6-8 weeks of age. During this period, we recorded the wheel revolutions in each 1-minute interval

over a period of 23 hours. We then computed 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 Jr. 2001; Hiramatsu and Garland Jr. 2018). A measure of wheel freeness was also included as a covariate in analyses of wheel running (e.g., Girard et al. 2007; Kolb et al. 2010). After the wheel-access period, mice were weighed, then group-housed (4 per cage by sex) until breeding pairs were established. In addition, for the offspring and grand-offspring generations, food consumption (with allowance for wastage (Koteja et al. 2003)) was measured as the change in food hopper weight over the 6-day wheel-access period.

Home-cage activity

Spontaneous physical activity was measured as home-cage activity, using a system described previously (Copes et al. 2015). At 5 weeks of age (i.e., prior to wheel-testing), 152 mice from the offspring generation were housed for a period of 3 days under a 12:12 light cycle in home cages fitted with a passive infrared sensor (Talon TL-Xpress-A; Crow Electronics, Fort Lee, New Jersey, USA), protected within a wire mesh. The sensors were connected to a computer with custom activity-recording software (developed by M.A. Chappell) via a digital I/O board (ICS 2313; ICS Electronics, Pleasanton, CA, USA). The

sensors recorded activity three times per second, and a mean value between 0 (no movement detected) and 1 (movement detected) was calculated for each minute over 23-hour periods of measurement. From these data, we obtained daily activity levels, duration of activity (number of minutes with activity detected), and average activity intensity (activity per minute). All analyses of home-cage activity used a measure of sensor sensitivity (taken 3 times before and after the testing period) as a covariate (Copes et al. 2015).

Open-field behavior

For the open-field measurements, two males and two females were randomly chosen from families within each line and wheel-access. For logistical purposes, these 148 mice were split into two measurement batches. We used a 100 x 100-cm arena with 50-cm-high walls constructed of five pieces of black Trovicel plastic held together with duct tape around the outside, as described in Bronikowski et al., (2001). Videos were recorded using an overhead Logitech HD C525 webcam placed above the arena. Trials were 5 minutes in duration and were performed in a dimly lit room (~7 lux on the floor of the testing arena). Given that mice are active and run on wheels primarily during the dark half of their light/dark cycle, we reversed the photoperiod of mice 2 weeks prior to testing. Within a measurement day, the order of mice was randomly assigned, and the time of day was noted. At the start of each trial, a mouse was grasped by the tail, minimizing any disturbance to the cage, and placed in the center of

the arena. The arena floor was wiped with a moist sponge (water only) between trials and allowed to dry before the next trial. We recorded the number of fecal boli and urine pools deposited on the arena floor during the testing period. Videos were later analyzed in a semi-automated fashion with the TopScan LITE video tracking software (Clever Sys, Inc.). A zone was digitally constructed and superimposed over the arena floor (e.g., area of Zone1 is equal to the surface area of the arena floor, i.e., 100 x 100-cm). Each of the following concentric zones was created as a proportion of the first zone corresponding to known distances of the physical arena: walls (Zone1, 41% of total area), outer (Zone2 is 76.5 x 76.5-cm, 28.27% of total area), middle (Zone3 is 55 x 55-cm, 24% of total area), center (Zone4 is 25 x 25-cm, 6.25% of total area), and limbo (defined as any period where the mouse was not tracked by the software). Trials were then analyzed automatically, where TopScan LITE recorded the following variables in 1-minute bins for each of the 5 distinct zones: time before the zone is entered for the first time (or for the center, the time before the mouse leaves), the number of bouts (i.e., times a mouse entered the zone), the time spent in the zone, the distance travelled, and the average speed. A diagram of these zones, as well as a representative trial, are presented in Figure 3. 7.

Organ masses

Two weeks after breeding (for males, ~20 weeks of age) and three weeks after weaning of their offspring (for females, ~24 weeks of age), mice were

euthanized via CO_2, tail length was measured, and the whole brain (which was photographed and subsequently partitioned into the cerebrum and cerebellum), heart (ventricles), liver, kidneys, spleen, triceps surae muscle group (left and right), reproductive fat (also termed epididymal fat pad in males and the ovarian and parametrial fat pads in females), and subdermal fat (also termed inguinal subcutaneous or posterior subdermal fat pads) (Cinti 2005; Bagchi and MacDougald 2019) were each collected and weighed. Photographs of the brain were processed using ImageJ, where measurements of the anterior-posterior length, left and right cerebral hemisphere areas (which were later combined to analyze total cerebrum area), and cerebellum area were taken (e.g., see Pucilowska et al. 2015).

Statistical analyses (Statistical model)

Statistical analyses were performed using SAS v15 (SAS Institute, Cary, NC, USA). The statistical model used (including fixed effects and SAS procedures) varied both within and across generations. However, in all cases, replicate line (4 HR and 4 C) was nested within linetype as a random effect such that the d.f. for linetype were always 1 and 6. Additionally, all other fixed effects (except for mini-muscle status, see below) were crossed within line (which is nested within linetype) as a random effect, such that the d.f. for all other fixed effects and interactions were also always 1 and 6. The following methods reflect the experimental timeline presented in Figure 3.1.

Maternal generation (g84): (1) The weaning and wheel testing periods were prior to the selection of breeding pairs for this experiment. Thus, traits were analyzed using SAS Proc MIXED with only linetype (HR vs C) and sex (female vs male) as fixed effects. Age and wheel freeness were used as covariates for wheel-running traits. (2) Data from maternal wheel-running were analyzed using SAS Proc MIXED as repeated measures, with an autoregressive covariance structure, and with linetype and day as fixed effects. Wheel running traits were analyzed using age and wheel freeness as covariates. (3) Data from maternal behavioral observations were analyzed using SAS Proc MIXED with linetype and maternal wheel access (wheels vs no wheels) as fixed effects. Age and litter size (which was a significant positive predictor of nursing behavior) were included as covariates. (4) Birth and weaning success were scored as 0 or 1, and analyzed using SAS GLIMMIX with linetype and wheel access as fixed effects. Age was also included as a covariate. Other traits measured at offspring weaning (e.g., litter size, sex ratio) were analyzed using SAS Proc MIXED with linetype and wheel access as fixed effects. Age was included as a covariate, and when analyzing litter size dam body mass was also included as a covariate.

Offspring generation (g85): All analyses were conducted using the same model, except for birth and weaning success, which were analyzed using SAS GLIMMIX (as described above). Offspring traits were analyzed using SAS Proc MIXED with linetype, sex, and wheel access as fixed effects. Home-cage activity were analyzed using a measure of sensor sensitivity as a covariate (age was

similar across individuals, and therefore was not used as a covariate). Data from the wheel testing period were analyzed using age and wheel freeness as covariates. Additionally, food consumption (measured during the wheel access period) was analyzed using body mass as a covariate. Food consumption was also analyzed using wheel running as a covariate with similar results. Open-field behavior was analyzed using time of day as a covariate. Again, age was similar across individuals, and so was not used as a covariate. Organ and tissue masses were analyzed with body mass, age, and time of day as covariates. Brain mass and digital tracing data were analyzed without body mass as covariates (e.g., see Schmill et al. 2023). 2-dimensional measurements of whole-brain, cerebrum, and cerebellum were analyzed using brain mass as a covariate. At several stages where body mass is routinely measured as part of the normal selection protocol (e.g., weaning, wheel testing), we also measured whole-body lean and fat mass with age and time of day as covariates. Analyses of fat mass also included lean mass as an additional covariate. Finally, all analyses were conducted again, with mini-muscle status as an additional main effect. Results from these analyses are reported separately.

Grand-offspring generation (g86): Analyses followed the procedures outlined above.

Least Squares Means (LSMs), associated 95% confidence limits, F-statistics, and p-values are reported for linetype, sex, maternal wheel access, and their interactions.

Statistical analyses (Outliers)

Prior to analysis, some values were removed due to known problems (e.g., equipment malfunction). Other values were removed via a series of logical tests (e.g., lean + fat mass cannot be greater than whole-body mass without substantial measurement error in one or more traits). Data were also graphed and visually inspected for potential outliers, which were subsequently removed if a source of error could not be found and corrected.

During statistical analyses, several criteria were used to determine potential outliers. First, data were sequentially removed if their standardized residuals were greater than ± 5, or ± 1 from the next closest standardized residual. If the absolute skew was less than ~0.3, analyses proceeded without further intervention. If absolute skew remained greater than ~0.3, we conducted a formal outlier test on the datum with the highest absolute standardized residual, as described in Cook and Weisberg (1999) and used in Belter et al. (2004), to determine whether the datum should be removed from analyses. If significant at $p < 0.05$, the datum was removed, and the outlier test was repeated. This continued until no more significant outliers were detected. If absolute skew remained greater than 0.3 after removing outliers, the data were transformed, and the outlier test was repeated as needed. If outliers were part of a series of data (e.g., lean, and fat mass are simultaneously measured using EchoMRI), they were removed from each analysis.

Statistical analyses (Multiple-comparison corrections)

In total, we analyzed 178 traits (73 from the maternal generation, 82 from the offspring generation, and 23 from the grand-offspring generation), resulting in 847 p-values (193 from the maternal generation, 530 from the offspring generation, and 124 from the grand-offspring generation) from main effects or interactions (but not covariates). Therefore, we used the SAS Procedure MULTTEST to implement the adaptive False Discovery Rate (aFDR) to control the FDR at 10%. Each generation constitutes a unique experimental cohort, and thus a different threshold was determined for each generation. The threshold for statistical significance with an adaptive FDR of 10% was 0.0131 for the maternal generation, 0.0030 for the offspring generation, and 0.0057 for the grand-offspring generation. P-values for main effects and interactions less than these thresholds (within their respective generations) are referred to as "significant" (indicated by **bold underline** in tables) and those with a nominal p-value less than 0.05 are considered “nominally significant" (indicated by **bold** in tables). Because the statistical power to detect interactions is lower than for detecting main effects (Wahlsten 1990), interactions with a nominal p-value < 0.10 are also considered “nominally significant" (also indicated by **bold** in tables), as we have done in other studies (e.g., see Cadney et al. 2022).

Results

Maternal wheel running before, during, and after pregnancy

Wheel-running data across all experimental phases are reported in Figure 3.2. During days 5 and 6 of the standard 6-day test to select breeders, females from HR lines ran more revolutions per day than those from C lines, as expected (+230%, $p < 0.0001$, Table 3.1: Wheel Testing, Figure 3.3A). The average number of revolutions run per day increased over the 6-day period ($P_{day} < 0.0001$) for all mice, but the per-day increase was larger for HR females than for C ($P_{linetype*day} < 0.0001$, Table 3.5).

Females from HR lines also ran more than those from C lines while housed individually for the two weeks prior to being paired with males (+182%, $p < 0.0001$) (Table 3.1: Maternal Wheel Access, Figure 3.2A). Again, the average number of revolutions run per day increased over the 14-day period, and the per-day increase was larger for HR prospective dams than for C ($P_{linetype*day} < 0.0001$, Table 3.5). When housed with males, running by HR mice was also substantially greater than for C mice (+117%, $p = 0.0004$), but we do not know which sex was doing the running, nor if the sex distribution of measured running might differ between HR and C mice (see Section 2.2). The average number of revolutions run per day decreased over this 18-day period ($P_{day} < 0.0001$), with the per-day decline being steeper for HR mice than for C ($P_{linetype*day} = 0.0659$, Table 3.5).

After males were removed, i.e., during the days leading up to parturition, HR dams ran more than C (+69%, $p = 0.0105$). The average number of

revolutions run per day continued to decrease over this period ($P_{day} < 0.0001$), again with a steeper decline for HR dams than for C ($P_{linetype*day} = 0.0334$, Table 3.5). After parturition (i.e., postpartum days 1-10), HR and C dams did not differ statistically in their running distance (+18%, p = 0.4585) (Table 3.1: Maternal Wheel Access, Figure 3.2A), although the number of revolutions run per day differed across the 10-day period ($P_{day} = 0.0051$, Table 3.5), with no interaction ($P_{linetype*day} = 0.6658$).

As noted in the Methods, we also analyzed the components of daily running distance. During days 5 and 6 of the standard 6-day test, females from HR lines ran faster (+152%, p < 0.0001) and longer (+25%, p = 0.0154) than C females (Table 3.1: Wheel Testing, Figures 3.3B and C). Females from HR lines also ran faster (+146%, p = 0.0006) and longer (+17%, p = 0.0365) than C when housed individually for two weeks prior to being paired with males. When housed as pairs, HR mice ran faster (+99%, p = 0.0003) but not longer (p = 0.4401) than C. After males were removed, HR dams again ran faster (+37%, p = 0.0247) but not longer (p = 0.2118) than C. After parturition, HR dams did not significantly run faster (p = 0.1647) or longer (p = 0.8633) than C (Table 3.1: Maternal Wheel Access, Figure 3.2B). Full statistical details for the repeated-measures analyses of running speed and duration can be found in Table 3.5.

Maternal wheel access had no significant effect on whole-body mass of dams at the end of the maternal wheel access period, i.e., 10 days post-partum (p = 0.5607, Table 3.1: Maternal Wheel Access, Figure 3.4A “Moms Off

Wheels”), nor whole-body (p = 0.5026), lean (p = 0.6404), or fat mass (p = 0.2768) at offspring weaning of dams, i.e., 21 days post-partum (Table 3.1: Figure 3.4A "Offspring Weaning").

Maternal behavior

Nursing accounted for ~87.5% of all maternal behavior observations, which was relatively consistent across experimental groups (the frequency of observed behaviors can be found in Table 3.6, Figure 3.5C). Additionally, maternal behavior was higher in the afternoon (when activity was lower; during the light cycle) and lower in the morning and evening (when activity was higher; during the dark cycle) for all groups (Table 3.6, Figure 3.5B).

Across all observations, dams with wheels had higher activity levels than those without (+16%, p = 0.0086). However, when wheel-running observations were removed, dams with wheels had lower activity levels than those without (-15%, p = 0.0100). HR and C dams did not significantly differ in overall activity (p = 0.1900) (Table 3.1: Maternal Behavior, Figure 3.5B). During post-partum days 1-5, HR and C dams with wheels had lower levels of maternal care (-13%, p = 0.0672), and higher levels of overall activity (+28%, p = 0.0603), than those without (Table 3.1: Maternal Behavior, Figure 3.5A). During post-partum days 6-10, linetype and wheel access interacted (p = 0.0218) to affect maternal care, wherein C dams with wheels had higher levels of maternal care than C dams without (+19%), while HR dams with wheels had lower levels of maternal care

than HR dams without (-15%) (Table 3.1: Maternal Behavior, Figure 3.3A). During post-partum days 11-15 and 16-20, following removal of wheels, there were no significant differences in activity or maternal care between HR and C dams or between those previously housed with and without wheels. However, during this period, there was a slight (+4%), although significant ($p = 0.0124$), delay in the average age that offspring from dams with wheels first opened their eyes (Table 3.1: Maternal Behavior).

Maternal reproductive success

We found no significant differences in the number of days from pairing breeders to offspring birth, birth success (i.e., did a dam give birth), or litter size based on linetype or maternal wheel access (Table 3.1: Offspring Weaning, Figure 3.6A). However, linetype and wheel access interacted ($p = 0.0447$) to affect weaning success, which was higher in C dams with wheels than those without (+21%), but lower in HR dams with wheels than those without (-16%) (Table 3.1: Offspring Weaning, Figure 3.6A). Additionally, the sex ratio at weaning (proportion of females to litter size) was higher in dams with wheel access (+20%, $p = 0.0129$), due to a decrease in the number of males ($p = 0.0363$) (Table 3.1: Offspring Weaning). In particular, the sex ratio of HR dams with wheel access was higher than that of any other group (+32%) (Table 3.1: Offspring Weaning).

Offspring and grand-offspring whole-body, lean, and fat mass

Offspring of dams with wheel access during pregnancy were smaller at weaning (-7%, $p = 0.0215$), due to a slight decrease in lean mass (-5%, $p = 0.0804$) (Table 3.2: Weaning, Figure 3.4B). This effect was also present around sexual maturity as linetype, where wheel access interacted to affect whole-body ($p = 0.0965$) and lean mass ($p = 0.0738$) prior to the standard 6-day period used to select breeder. HR offspring from dams with wheels were smaller than those without (-4%), but C offspring did not differ (+0.3%) (Table 3.1: Offspring Weaning, Figure 3.4B). However, neither offspring nor grand-offspring of dams with wheel access differed significantly from those of dams without wheels in whole-body, lean, or fat mass in a consistent manner (Tables 3.2 and 3.3, Figures 3.4B and 3.4C).

Offspring and grand-offspring wheel running

In each generation, HR lines ran more than C lines (+230% and $p < 0.0001$ in parental generation; +210% and $p < 0.0001$ in offspring, +224% and $p < 0.0001$ in grand-offspring) by running faster (+152% and $p < 0.0001$ in parental generation; +129% and $p < 0.0001$ in offspring; +135% and $p < 0.0001$ in grand-offspring) and for more minutes per day (+35% and $p = 0.0154$ in parental generation; +38% and $p = 0.0252$ in offspring; +35% and $p = 0.0426$ in grand-offspring) during the standard 6-day test to choose breeders (Tables 3.1-3.3: Wheel Testing, Figure 3.3). Across each generation, females ran more than

males (+21% and p = 0.0006 in parental generation; +38% and p = 0.0154 in offspring; +20% and p = 0.0539 in grand-offspring) by running for longer (+19% and p = 0.0006 in parental generation; +28% and p = 0.0013 in offspring; +21% and p = 0.0012 in grand-offspring), but generally not faster (+7% and p = 0.1225 in parental generation; +11% and p = 0.0339 in offspring; +5% and p = 0.2456 in grand-offspring) (Tables 3.1-3.3: Wheel Testing, Figure 3.3).

Wheel access and sex interacted to affect wheel-running distance (p = 0.0683) and duration (p = 0.0926), but not speed (p = 0.3905) in the offspring generation only, such that female offspring of dams with wheels ran fewer revolutions per day (-12%) and fewer minutes per day (-10%) than offspring of dams without wheels. In contrast, male offspring of dams with wheels ran more revolutions per day (+6%) and more minutes per day (+3%) than those of dams without wheels (Table 3.2: Wheel Testing, Figure 3.3A-D).

Offspring and grand-offspring reproductive success

We found no significant effects of linetype or wheel access on the number of days from pairing breeders to parturition, birth success, weaning success, litter size, or sex ratio, in offspring or grand-offspring generations.

Offspring home-cage activity

Home-cage activity (measured in the absence of wheels at ~36 days of age) was higher in HR than C lines (+43%, p = 0.0029), due to both more active

minutes per day (+18%, p = 0.0158) and more activity per minute (+20%, p = 0.0143) (Table 3.2: Home-Cage Activity). Females were also more active than males (+22%, p = 0.0193), due to a higher activity per minute (+20%, p = 0.0369), but not more active minutes per day (+7%, p = 0.1718) (Table 3.2: Home-Cage Activity).

Offspring open-field behavior

Across all groups, mice spent ~81% of the open-field trial near the walls (Table 3.2: Open-Field Behavior). Overall, we found no significant differences based on linetype or maternal wheel access in the number of fecal boli or urine pools, the latency to exit the center zone, the latency to enter the wall zone; the time spent, average speed, or distance travelled near the walls; the overall average speed, or total distance travelled (Table 3.2: Open-Field Behavior).

However, HR mice tended to run more slowly than C mice (-33%, p = 0.0591), and thus traversed less distance overall (p = 0.0790), in the 1st minute of the open-field trial. HR mice also ran more slowly than C mice near walls during the 1st minute (-31%, p = 0.0374), but did not differ in the distance travelled near walls (p = 0.2299) (Table 3.2: Open-Field Behavior). Linetype and sex interacted to affect average running speed, and thus total distance, in the 1st, 2nd, and 3rd minutes (p = 0.0425 in the 1st minute; p = 0.0419 in the 2nd minute; p = 0.0905 in the 3rd minute), where C females ran more slowly (-19% in the 1st minute; -12% in the 2nd minute; -14% in the 3rd minute), and thus traversed less distance, than

their male counterparts, while HR females ran faster (+11% in the 1st minute; +25% in the 2nd minute; +11% in the 3rd minute), and thus traversed more distance, than their male counterparts (Table 3.2: Open-Field Behavior). Linetype and sex also interacted to affect the average speed near walls in the 1st and 2nd minutes ($p = 0.0245$ in the 1st minute; $p = 0.0355$ in the 2nd minute), where C females ran slower (-17% in the 1st minute; -13% in the 2nd minute) than their male counterparts, while HR females ran faster (+23% in the 1st minute; +27% in the 2nd minute) than their male counterparts (Table 3.2: Open-Field Behavior).

Offspring tissue masses

With body mass and age as covariates, masses of heart ventricle, liver, spleen, and subdermal fat pads were not significantly affected by linetype, sex, or maternal wheel access (Table 3.2: Dissection). However, mice from HR lines had larger brains than those from C lines (+8%, $p = 0.0299$), due to having a larger cerebrum ($p = 0.0298$) but not a larger cerebellum ($p = 0.1334$) (Table 3.2: Dissection). Kidney and reproductive fat pads (i.e., epididymal fat pads in males and a combination of the ovarian and parametrial fat pads in females) were smaller in females than in males (-30%, $p = 0.0009$ and -79%, $p = 0.0029$ respectively) (Table 3.2: Dissection).

Discussion

We tested two hypotheses using mice from a long-term artificial selection experiment for high voluntary wheel-running behavior: (1) maternal exercise before, during, and after pregnancy could improve indicators of offspring health (e.g., adult activity levels, body composition); (2) maternal exercise during the post-partum period may negatively impact offspring, either via (a) introducing conflict between competing biological “drives" (i.e., maternal care versus voluntary exercise), and/or (b) dams from the selectively bred High Runner (HR) lines may engage in “excessive” activity that could result in detrimental effects on offspring, either of which could have adverse effects on offspring growth and development

Minimal effects on offspring physical activity and body composition

Maternal wheel access had few statistically significant effects on offspring, and no significant effects on grand-offspring traits. We view this as particularly surprising for HR dams, given their possibly excessive running behavior (Table 3.1: Maternal Wheel Access, Figure 3.2). However, maternal wheel access did cause a developmental delay, as indicated by eye opening (Table 3.1: Maternal Behavior), a key transitionary period in the maturation of the visual cortex in mice (Shen and Colonnese 2016). Additionally, maternal wheel access resulted in reduced offspring lean mass both at weaning and immediately prior to wheel access (Table 3.2: Weaning and Wheel Testing, Figure 3.4B). We speculate that

maternal exercise may have, through mechanisms not investigated in the present study, induced low levels of intrauterine growth restriction, causing the developmental delay and smaller offspring body size. Specifically, maternal wheel access may have caused changes related to hormones associated with the regulation of nutrient availability, such as leptin (see Section 4.5: Future Directions).

The reduced mass of offspring whose mothers had wheel access recovered by adulthood (Table 3.2, Figure 3.4B). This is perhaps unsurprising, as mammalian compensatory growth is a well-established phenomenon (Wilson and Osbourn 1960). Compensatory growth, also termed adaptive growth plasticity, is a period of accelerated growth that may occur after a period of slowed development, especially if due to nutrient deprivation. This adaptive growth plasticity allows offspring to partly compensate for reduced maternal investment (and other developmental constraints), but only as offspring become more nutritionally independent (e.g., after weaning) (Berghänel et al. 2017). In the present study, offspring were provided food and water *ad libitum* throughout their lives, and thus likely had ample opportunity for compensatory growth to occur.

Competing biological "drives" reduce maternal care and offspring survival in HR lines

We hypothesized that dams -- especially HR dams -- given a choice between competing “drives” (Stults-Kolehmainen 2023), i.e., voluntary exercise and maternal care, may behave and allocate resources differently than when “unchallenged” (e.g., see Zhao et al. 2018). Our results for HR dams with wheels support this hypothesis as they consistently spent more time engaging in activity behaviors and less time engaging in maternal care throughout postpartum days 1-5 and 6-10 (Table 3.1: Maternal Behavior, Figure 3.5). In contrast, C dams with wheels varied in their maternal care over the same period, with lower levels (compared to C dams without wheels) during post-natal days 1-5 but relatively higher levels during post-natal days 6-10 (Table 3.1: Maternal Behavior, Figure 3.5). Without wheels, HR and C dams did not differ statistically in maternal care behavior.

For both HR and C dams, differences in maternal care were primarily due to a reduced time spent nursing (Table 3.1: Maternal Care, Figure 3.5C). This reduced nursing may have been due to an inability to simultaneously support wheel running, an energetically challenging activity (Koteja et al. 1999; Swallow et al. 2001; Rezende et al. 2009; Copes et al. 2015), and the high energetic cost that nursing imposes (e.g., for mice see Hammond and Diamond 1992; National Research Council (US) Subcommittee on Laboratory Animal Nutrition 1995; Hammond 1997; e.g., for humans see Butte and King 2005). Given that offspring utilize milk for weight gain most efficiently around post-natal day 7 (Knight et al., 1986) (i.e., prior to wheel removal in the present study), a reduction in nursing or

nutrient availability at such a critical period may have disrupted development (e.g., as indicated by delayed eye opening in our study). In fact, reductions in various aspects of maternal care (e.g., nursing, grooming pups) have been shown to adversely affect offspring health (e.g., increased anxiety-like behavior in mice, Pedersen et al. 2017 and citations therein), future maternal behavior (in rats, Curley and Champagne 2016 and citations therein), and survival (e.g., in domestic mammals, Nowak et al. 2000; including Théoret-Gosselin et al. 2015). Although we did not observe any lasting effects on offspring (see above), the aforementioned changes in the amount and timing of maternal care may have caused differential success in rearing offspring to weaning, despite similar success giving birth (Table 3.1: Offspring Weaning, Figure 3.6). We propose that: (1) as compared with post-natal days 1-5, maternal care behavior during post-natal days 6-10 may have a larger influence on the eventual survival of offspring to weaning, (2) HR dams with wheels were less successful than those without, and least successful overall, because of their consistently lower levels of maternal care behavior throughout post-natal days 1-5 *and* 6-10, and (3) C dams with wheels were more successful than those without, and most successful overall, because of their relatively high levels of maternal care during post-natal days 6-10 (Table 3.1: Offspring Weaning, Figure 3.6A).

Although several previous studies have examined the effects of maternal exercise on offspring characteristics in rodents (e.g., see reviews in Blaize et al. 2015; Kusuyama et al. 2020), few have tested the effects of exercise opportunity

on maternal care behavior. Those that have tend to show no effect or improved maternal care behavior with maternal exercise. For example, maternal swimming exercise for 4 weeks, starting one week before mating (5 days/week, 30 minutes/session), did not result in any statistically significant changes to maternal care behavior (observed 5 times/day from post-natal days 2-9) in rats (August et al. 2021). However, NMRI mice provided wheel access from conception to post-natal day 7 had increased levels of maternal care (observed 3 times/day during the first two days post-partum) (Naghibi et al. 2022). Thus, the decreased maternal care behavior observed in the genetically diverged HR lines (Hillis et al. 2020; Hillis and Garland Jr. 2023) when given wheel access is consistent with other studies showing that genetic background affects maternal care among strains of mice (Champagne et al. 2007) and also with our suggestion that HR dams' motivation for wheel running is high enough to cause competition with care of offspring.

Notably, changes in the brain's reward circuitry are thought to underlie higher motivation for voluntary physical activity in HR mice. Evidence for changes to the reward system include differential sensitivity of wheel running to dopamine reuptake transport blockers (Rhodes et al. 2001, 2005; Rhodes and Garland Jr. 2003) and endocannabinoid agonists and antagonists (Keeney et al. 2008, 2012), differences in circulating endocannabinoid levels (Thompson et al. 2017), and differential activation of the reward circuit during withdrawal from wheels (Rhodes et al. 2003; Saul et al. 2017), in addition to behavioral

reinforcement responses (Belke and Garland Jr. 2007). However, changes in dopamine signaling and activation of the reward circuit are often shared across forms of motivation and reward. Consistent with this idea, HR mice consume less artificial sweetener than C, but only when housed with wheels, which may be attributed to the stronger reward of wheel running that has evolved in HR mice (Thompson et al. 2018). On the other hand, a recent study of conditioned place preference using cocaine and methylphenidate (Ritalin) did not find any conditioning differences between HR and C females (Schmill et al. 2021). We note here that although humans may or may not possess a similar “drive” for physical activity (Stults-Kolehmainen 2023), we do seem to have several neurobiological mechanisms linking exercise and health (Raichlen and Polk 2013; Raichlen and Alexander 2017). Of particular relevance for the present study, insufficient exercise during pregnancy (Walasik et al. 2020) may lead to several metabolic ailments in offspring (e.g. obesity, type II diabetes) later in ontogeny (Blaize et al. 2015 and citations therein), and thus modern inadequate exercise, including during pregnancy, may cause a pathology as a result of the mismatch between our evolutionary history and current environment (Gluckman and Hanson 2004; Waterland and Michels 2007; Mattson 2012; Pontzer et al. 2018).

Maternal exercise results in female-biased litters at weaning

Interestingly, dams with wheels had litters whose sex ratio significantly favored females (Table 3.1: Offspring Weaning), especially in HR dams (+14% in HR dams vs +4% in C dams, $P_{linetype*wheel} = 0.1024$). Although some avian species can adjust their offspring sex ratio in a consistent manner that seems to be adaptive based on environmental conditions (Nager et al. 1999; West and Sheldon 2002; West et al. 2002), the notion that mammals can similarly modify the sex ratio of their offspring in a consistent and adaptive manner is controversial (Rosenfeld et al. 2003). However, there is evidence of variation in the sex ratio of mammals at birth due to food availability or competition for resources (Clutton-Brock and Iason 1986; Wild and West 2007), and major changes in the sex ratio of offspring have been reported for various species of deer (Clutton-Brock et al. 1997; Kruuk et al. 1999).

Sex allocation theory, which began with Trivers and Willard (1973), posits that: (1) when a small proportion of males sire a large proportion of offspring but do not invest in their care, (2) females in good body condition are expected to produce more males than females, as high investment in male offspring would increase their ability to join the small proportion of breeding males, and (3) females in poor body condition are expected to produce more females than males, as their inability to invest in male offspring would decrease their ability to join the small proportion of breeding males. Predictions derived from this theory have met with variable success when applied to wild mammalian populations,

potentially due to factors other than maternal body condition, e.g., population density (Kruuk et al. 1999). However, food-restriction studies using rodents often produce female-biased litters (Labov et al. 1986; Meikle and Drickamer 1986; Wright et al. 1988; Meikle and Thornton 1995; Rosenfeld et al. 2003; Rosenfeld and Roberts 2004), as do other stressors (Krackow 1997; Firman 2020), which could be interpreted as support for sex allocation theory.

With regard to the HR mouse model, under the routine selection protocol, only 1 of 5 males in each family sire offspring, and in HR lines only the highest-running male is chosen, which could qualify as an "elite pool" as described by Trivers and Willard (1973). Additionally, males do not invest in the care of offspring, as they are removed prior to offspring birth. Although we did not measure food consumption nor body composition of dams during the maternal wheel-access period, wheel running is energetically taxing (especially for HR mice), and as proposed above, may have induced enough of an energetic challenge to reduce nursing (i.e., dams with wheels may have been in "poor body condition"). An energetic challenge in dams with wheels, within the context of the sex allocation theory, could provide a possible explanation for the female-biased litters observed herein, and perhaps supports a selective prenatal vulnerability of male fetuses observed in many mammals, including humans (Crawford et al. 1987; Andersson and Bergström 1998; McCarthy 2019; Sutherland and Brunwasser 2018). Although we did not measure sex ratio at birth, a study at

generation 20 found no difference between the HR and C lines (Girard et al. 2002).

Comparisons with other HR mouse studies

The present study slightly differs in protocol from the ~30 years of selective history for running behavior in that wheel access was provided for the entire breeding period, rather than only the 6-day period used to select breeders. That said, our results are consistent with those found in other experiments using the HR mouse model (Hiramatsu and Garland Jr. 2018). For example, HR lines continue to run ~3-fold more revolutions per day than C lines (Tables 3.1-3.3: Wheel Testing, Figure 3.3A). Higher daily running was achieved by increases in both average speed (Tables 3.1-3.3: Wheel Testing, Figure 3.3C) and duration (Tables 3.1-3.3: Wheel Testing, Figure 3.3B). In fact, throughout all experimental phases, differences in wheel running between HR and C lines were due to changes to running speed, not duration, which matches how increased wheel running in HR females has primarily evolved (Swallow et al. 1998; Garland Jr. et al. 2011a; Claghorn et al. 2017; Copes et al. 2018; Schwartz et al. 2023). Additionally, HR mice increase their daily running over the 6-day period by larger amounts than C mice, evidenced by a sharper incline in the daily running distance ($P_{linetype * day} < 0.0001$), which differs from an analysis of HR mice at generation 21 (Kelly et al. 2006).

Several tests performed within this study have also been conducted in other experiments using the HR mouse model, with similar results. For example, we did not find any statistically significant differences based on linetype for maternal care behavior when dams were housed without wheels (Table 3.1: Maternal Behavior, Figure 3.5), consistent with prior studies (Girard et al. 2002; Keeney 2011; Hiramatsu et al. 2017). Additionally, there were no statistically significant differences based on linetype for birth success, wean success, litter size, or sex ratio (Tables 3.1-3.3: Offspring Weaning, Figure 3.6), as reported previously (Girard et al. 2002; although not in all generations, e.g., see Keeney 2011). Food consumption during the 6-day wheel-access period was higher in HR lines (Tables 3.2 and 3.3: Wheel Testing), but this difference was not statistically significant after accounting for daily running distance, as seen in Copes et al. (2015). HR lines were more active in home cages (Table 3.2: Home-Cage Activity), measured at 5 weeks of age and without access to wheels, due to a higher number of active periods and higher activity per minute, also found in other studies (Malisch et al. 2009; Copes et al. 2015; Hiramatsu et al. 2017 p. 201; Cadney et al. 2022). We found no statistically significant differences in open-field distance travelled, speed, or duration spent near walls (Table 3.2: Open-Field Behavior), similar to what was found in previous studies (Bronikowski et al. 2001; Careau et al. 2012; Cadney et al. 2021). Finally, of the organs measured, only brain mass differed statistically significantly between HR and C lines (Table 3.2: Dissection), consistent with previous studies that

demonstrate HR mice have larger brains (Kolb et al. 2013; Schmill et al. 2023). Overall, our data are comparable to that which is often collected as part of the broader selection experiment.

Concluding Remarks and Future Directions

A key aspect of the present study was to evaluate the behavior of dams provided the opportunity to choose between competing biological "drives," maternal care and voluntary exercise. Thus, aspects of the experimental design were structured around minimizing potential disturbances that might otherwise have altered the behavior of dams. However, as a result we were unable to collect other data that, in hindsight, may have provided valuable information.

For example, we found that maternal wheel access resulted in smaller offspring at weaning, but because we did not measure litter characteristics at birth, we were unable to differentiate between potential effects of wheel running on the in-utero environment experienced by offspring and the effects of decreased nursing behavior observed in the presence of wheels. Regarding the former, studies in humans have shown that routine aerobic exercise during pregnancy can result in smaller offspring at birth (Hopkins et al. 2011). Human mothers who exercised also had elevated circulating leptin (Hopkins et al. 2011; for another example, see Andersson-Hall et al. 2021), leading some to propose that elevated leptin may play a role in regulating offspring growth in utero (Pérez-Pérez et al. 2018; de Knegt et al. 2021). Typically, leptin is secreted by adipose

tissue and is thought to signal relative fat stores (i.e., energetic stores) to the brain. However, during pregnancy the placenta is also a major source of leptin production (Masuzaki et al. 1997; Highman et al. 1998). Although the exact mechanism is unknown, it is thought that exercise-induced changes to the placenta may alter placental leptin levels (Chae et al. 2022), and thus maternal circulating leptin. In this case, elevated placental leptin may disrupt placental growth and development through such key processes as cell proliferation and turnover (Pérez-Pérez et al. 2018 and citations therein), thus limiting nutrient availability for offspring. If true, the above would provide an indirect mechanism wherein maternal exercise can be linked to offspring development in utero. Although we did not measure circulating leptin in the present study, adult female HR mice (measured without wheels and not during pregnancy) have lower circulating leptin concentrations, even after adjusting for their lower body fat (Girard et al. 2007 p. 200; Meek et al. 2012; Acosta et al. 2015). It is unclear what effect, if any, a lower baseline level of leptin may have had on the effect of maternal exercise on circulating leptin levels during pregnancy. Future research regarding the potential effects of exercise during pregnancy on relevant hormones (e.g., leptin) should be explored using the HR mouse model.

As a final note, when dams and sires were paired for 18 days, both sexes were likely running on wheels (see Section 2.2). As a result, sperm may have been affected in a way that could influence offspring characteristics. For example, in C57BL6 mice, 8 weeks of swimming exercise (30-minutes, 3 times

per week) for obese males before they were paired for breeding improved the metabolic health of their female offspring (McPherson et al. 2015). Additionally, also in C57BL6 mice, 4 weeks of voluntary wheel running for males before they were paired for breeding reduced indicators of conditioned fear and anxiety in their male offspring, but did not affect female offspring (Short et al. 2017). Thus, male exercise prior to conception could have influenced the results of the present study, though there would be less time for sperm to undergo exercise-induced changes when females conceived earlier (most females conceived 1-3 days after males were added).

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Table 3.1. Least Squares Means (LSMs), F-statistics, and p-values from analyses conducted on the maternal generation. Some data were transformed prior to analysis, then back-transformed for LSMs. This procedure requires the computation of 95% Confidence Limits (95% CL), rather than Standard Errors (SE); therefore, we calculated Upper and Lower 95% CL (UL and LL respectively) for all traits, regardless of whether data were transformed or not. See section 2.10 for a complete description of statistical analyses used. In brief, all analyses were performed using SAS v15, although the statistical model used (including fixed effects and SAS procedures) varied. In all cases, replicate line (4 HR and 4 C) was nested within linetype as a random effect. Degrees of freedom for testing the effects of linetype, wheel access, and their interaction were always 1 and 6. As described in section 2.12, p-values are not corrected for multiple comparisons. For main effects, nominal $p < 0.05$ are indicated by **bold**; for interactions, nominal $p < 0.10$ are also indicated by **bold**. For both main effects and interactions, p-values that remain significant while controlling for multiple comparisons with an adaptive FDR of 10% are indicated by **bold and underline**. For traits measured before the start of the maternal wheel access period (i.e., weaning and wheel testing), females and males are designated as “no wheel” for accurate comparison with offspring and grand-offspring generations, given “no wheel” corresponds to no maternal wheel access.

Table 3.1. Continued.

	Variable	Linetype		Sex		Wheel Access		Linetype* Wheel Access		Linetype* Sex	
		F	P	F	P	F	P	F	P	F	P
Weaning	Body mass (g)	1.20	0.3152	34.11	**0.0011**					9.61	**0.0211**
Wheel Testing	Body mass before wheels (g)	3.41	0.1143	295.14	**< 0.0001**					0.90	0.3806
	Distance (revs/day)	247.88	**< 0.0001**	42.57	**0.0006**					6.02	**0.0495**
	Duration (min)	11.22	**0.0154**	41.93	**0.0006**					5.24	**0.0621**
	Avg. speed (revs/min)	88.34	**< 0.0001**	3.23	0.1225					1.62	0.2502
	Max. speed (revs/min)	111.44	**< 0.0001**	1.97	0.2103					2.40	0.1726
	Body mass after wheels (g)	2.48	0.1666	179.85	**< 0.0001**					2.44	0.1690
Maternal Wheel Access	Body mass before wheels (g)	2.79	0.1460								
	Distance, before breeding (revs/day)	89.86	**< 0.0001**								
	Duration, before breeding (min)	7.18	**0.0365**								
	Avg. speed, before breeding (revs/min)	42.96	**0.0006**								
	Distance, dams + sires (revs/day)	48.74	**0.0004**								
	Duration, dams + sires (min)	0.68	0.4401								
	Avg. speed, dams + sires (revs/min)	52.57	**0.0003**								
	Distance, during pregnancy (revs/day)	13.42	**0.0105**								
	Duration, during pregnancy (min)	1.95	0.2118								
	Avg. speed, during pregnancy (revs/min)	8.87	**0.0247**								
	Distance, post-partum (revs/day)	0.63	0.4585								
	Duration, post-partum (min)	0.03	0.8633								
	Avg. speed, post-partum (revs/min)	2.50	0.1647								
	Body mass after wheels (g)	1.50	0.2671			0.38	0.5607	2.00	0.2071		
Maternal Behavior	Maternal care, days 1-5	0.26	0.6286			4.98	0.0672	0.30	0.6043		
	Maternal care, days 6-10	0.07	0.8051			0.00	0.9545	9.46	**0.0218**		
	Maternal care, days 11-15	0.76	0.4161			0.35	0.5743	0.50	0.5073		
	Maternal care, days 16-20	0.33	0.5880			0.33	0.5847	0.93	0.3730		
	Maternal care, all days	0.02	0.9024			1.44	0.2758	1.96	0.2113		
	Maternal care, mornings	0.08	0.7893			0.04	0.8515	1.07	0.3401		
	Maternal care, afternoons	1.15	0.3247			0.52	0.4989	0.43	0.5386		
	Maternal care, evenings	0.51	0.5002			1.61	0.2518	2.25	0.1846		
	Nursing, days 1-5	0.15	0.7115			4.15	0.0879	0.41	0.5437		
	Nursing, days 6-10	0.06	0.8102			0.06	0.8103	9.81	**0.0203**		
	Nursing, days 11-15	0.11	0.7544			0.04	0.8400	0.09	0.7702		
	Nursing, days 16-20	0.50	0.5072			0.26	0.6259	0.12	0.7438		
	Nursing, all days	0.14	0.7212			0.74	0.4234	2.41	0.1716		
	Nursing, mornings	0.00	0.9648			0.11	0.7539	1.88	0.2197		
	Nursing, afternoons	2.45	0.1688			0.04	0.8555	0.49	0.5113		
	Nursing, evenings	0.15	0.7126			0.84	0.3955	1.29	0.2997		
	Activity, days 1-5	0.12	0.7395			5.34	0.0603	0.00	0.9866		
	Activity, days 6-10	2.22	0.1870			10.98	**0.0161**	0.05	0.8237		
	Activity, all days	2.18	0.1900			14.68	**0.0086**	0.00	0.9988		
	Activity, mornings	1.45	0.2734			5.36	0.0599	0.41	0.5449		
	Activity, afternoons	0.74	0.4242			1.82	0.2264	0.34	0.5791		
	Activity, evenings	4.54	0.0772			11.16	**0.0156**	0.02	0.9059		
	Non-wheel activity, days 1-5	0.12	0.7365			25.36	**0.0024**	1.16	0.3231		
	Non-wheel activity, days 6-10	3.22	0.1229			16.02	**0.0071**	1.15	0.3248		
	Non-wheel activity, days 11-15	3.28	0.1203			1.42	0.2778	0.32	0.5924		
	Non-wheel activity, days 16-20	2.61	0.1570			0.35	0.5764	0.35	0.5782		
	Non-wheel activity, all days	3.01	0.1334			13.75	**0.0100**	0.72	0.4277		
	Non-wheel activity, mornings	1.77	0.2318			5.71	0.0541	1.00	0.3556		
	Non-wheel activity, afternoons	0.87	0.3863			0.46	0.5244	0.52	0.4974		
	Non-wheel activity, evenings	6.86	**0.0396**			16.85	**0.0063**	1.43	0.2768		
	Maintenance, days 1-5	0.56	0.4838			0.12	0.7442	0.76	0.4162		
	Maintenance, days 6-10	5.79	0.0529			15.01	**0.0082**	12.11	**0.0131**		
	Maintenance, days 11-15	1.75	0.2335			5.86	0.0519	0.15	0.7139		
	Maintenance, days 16-20	6.53	**0.0432**			0.00	0.9527	0.04	0.8445		
	Maintenance, all days	4.64	0.0748			8.42	**0.0273**	3.34	0.1173		
	Maintenance, mornings	4.03	0.0915			7.60	**0.0330**	0.73	0.4254		
	Maintenance, afternoons	0.84	0.3942			0.00	0.9839	0.98	0.3599		
	Maintenance, evenings	6.65	**0.0419**			6.71	**0.0412**	4.14	**0.0882**		
	Offspring first eyes open	2.40	0.1725			12.44	**0.0124**	0.00	0.9492		
	Offspring first movement	0.85	0.3920			1.39	0.2831	0.44	0.5320		
	Offspring first solid food consumption	1.04	0.3467			3.37	0.1159	0.00	0.9691		
Offspring Weaning	Body mass (g)	1.21	0.3135			0.51	0.5026	2.10	0.1970		
	Lean mass (g)	1.59	0.2539			0.24	0.6404	5.61	**0.0557**		
	Fat mass (g)	0.36	0.5684			1.43	0.2768	0.00	0.9491		
	Pairing to offspring birth (days)	0.18	0.6835			0.12	0.7429	2.60	0.1581		
	Birth success (%)	0.57	0.4779			1.95	0.2118	0.04	0.8566		
	Wean success, of all dams (%)	0.43	0.5360			0.12	0.7369	3.99	**0.0929**		
	Wean success, of births (%)	0.50	0.5074			0.22	0.6570	6.40	**0.0447**		
	Litter size	3.13	0.1274			0.09	0.7774	0.00	0.9524		
	Sex ratio (# of females/total)	2.34	0.1769			12.24	**0.0129**	3.71	0.1024		

Table 3.1. Continued.

	Variable	Control											
		Female						Male					
		No Wheel			Wheel			No Wheel			Wheel		
		LSM	LL	UL	LSM	LL	UL	LSM	LL	UL	LSM	LL	UL
Weaning	Body mass (g)	12.51	10.50	14.51				13.30	11.29	15.31			
Wheel Testing	Body mass before wheels (g)	25.38	22.93	27.84				31.78	29.32	34.23			
	Distance (revs/day)	4975	4078	5872				3838	2938	4738			
	Duration (min)	498.3	416.0	580.6				375.1	292.7	457.6			
	Avg. speed (revs/min)	9.946	8.296	11.67				9.660	8.020	11.37			
	Max. speed (revs/min)	19.45	17.38	21.57				19.55	17.48	21.68			
	Body mass after wheels (g)	25.18	22.50	27.86				31.93	29.25	34.61			
Maternal Wheel Access	Body mass before wheels (g)				27.07	24.59	29.56						
	Distance, before breeding (revs/day)				5640	4724	6555						
	Duration, before breeding (min)				471.7	430.6	512.9						
	Avg. speed, before breeding (revs/min)				11.73	10.16	13.30						
	Distance, dams + sires (revs/day)				6030	5401	6658						
	Duration, dams + sires (min)				623.3	555.5	691.1						
	Avg. speed, dams + sires (revs/min)				9.714	8.948	10.49						
	Distance, during pregnancy (revs/day)				3141	2330	3951						
	Duration, during pregnancy (min)				316.6	252.9	380.3						
	Avg. speed, during pregnancy (revs/min)				9.370	7.618	11.12						
	Distance, post-partum (revs/day)				1165	794.3	1653						
	Duration, post-partum (min)				142.7	110.4	179.1						
	Avg. speed, post-partum (revs/min)				8.937	6.509	11.54						
	Body mass after wheels (g)	42.01	37.63	46.38	42.50	38.14	46.86						
Maternal Behavior	Maternal care, days 1-5	0.5819	0.4895	0.6743	0.5213	0.4289	0.6137						
	Maternal care, days 6-10	0.4047	0.3018	0.5076	0.4831	0.3803	0.5859						
	Maternal care, days 11-15	0.4839	0.4081	0.5597	0.5209	0.4450	0.5968						
	Maternal care, days 16-20	0.4549	0.3747	0.5351	0.4209	0.3410	0.5008						
	Maternal care, all days	0.4827	0.4131	0.5523	0.4860	0.4164	0.5556						
	Maternal care, mornings	0.4515	0.3160	0.5870	0.4747	0.3393	0.6101						
	Maternal care, afternoons	0.7562	0.6801	0.8323	0.7544	0.6783	0.8305						
	Maternal care, evenings	0.2397	0.1752	0.3042	0.2454	0.1818	0.3090						
	Nursing, days 1-5	0.5314	0.4433	0.6195	0.4891	0.4011	0.5771						
	Nursing, days 6-10	0.3925	0.2879	0.4971	0.4620	0.3576	0.5664						
	Nursing, days 11-15	0.4554	0.3700	0.5408	0.4724	0.3871	0.5577						
	Nursing, days 16-20	0.2696	0.1802	0.3590	0.2729	0.1837	0.3621						
	Nursing, all days	0.4130	0.3461	0.4799	0.4234	0.3566	0.4902						
	Nursing, mornings	0.3704	0.2527	0.4984	0.3972	0.2771	0.5272						
	Nursing, afternoons	0.6496	0.5678	0.7281	0.6634	0.5824	0.7413						
	Nursing, evenings	0.2113	0.1508	0.2718	0.2165	0.1568	0.2762						
	Activity, days 1-5	0.2897	0.2061	0.3733	0.3694	0.2858	0.4530						
	Activity, days 6-10	0.2513	0.1737	0.3354	0.3224	0.2392	0.4115						
	Activity, all days	0.2817	0.2100	0.3534	0.3334	0.2617	0.4051						
	Activity, mornings	0.2827	0.1603	0.4051	0.3239	0.2015	0.4463						
	Activity, afternoons	0.0992	0.0606	0.1419	0.1236	0.0827	0.1683						
	Activity, evenings	0.4588	0.3682	0.5494	0.5365	0.4469	0.6261						
	Non-wheel activity, days 1-5	0.2932	0.2135	0.3729	0.1157	0.0360	0.1954						
	Non-wheel activity, days 6-10	0.2551	0.1802	0.3359	0.1524	0.0883	0.2242						
	Non-wheel activity, days 11-15	0.2330	0.1365	0.3295	0.2521	0.1556	0.3486						
	Non-wheel activity, days 16-20	0.3432	0.2277	0.4587	0.3762	0.2607	0.4917						
	Non-wheel activity, all days	0.2829	0.2145	0.3513	0.2274	0.1591	0.2957						
	Non-wheel activity, mornings	0.2806	0.1652	0.3960	0.2085	0.0933	0.3237						
	Non-wheel activity, afternoons	0.0992	0.0613	0.1411	0.1161	0.0767	0.1592						
	Non-wheel activity, evenings	0.4582	0.3714	0.5450	0.3460	0.2600	0.4320						
	Maintenance, days 1-5	0.1170	0.0708	0.1685	0.0952	0.0517	0.1445						
	Maintenance, days 6-10	0.3299	0.2554	0.4089	0.1716	0.1097	0.2397						
	Maintenance, days 11-15	0.2761	0.1909	0.3686	0.2095	0.1308	0.2966						
	Maintenance, days 16-20	0.3359	0.2525	0.4193	0.3392	0.2558	0.4226						
	Maintenance, all days	0.2350	0.1823	0.2908	0.1771	0.1284	0.2295						
	Maintenance, mornings	0.2672	0.2049	0.3295	0.1997	0.1374	0.2620						
	Maintenance, afternoons	0.1288	0.0639	0.2034	0.1040	0.0436	0.1754						
	Maintenance, evenings	0.3051	0.2432	0.3670	0.2197	0.1583	0.2811						
	Offspring first eyes open	13.84	13.13	14.55	14.45	13.74	15.16						
	Offspring first movement	13.02	11.71	14.19	13.69	12.47	14.80						
	Offspring first solid food consumption	16.40	15.70	17.10	16.95	16.25	17.65						
Offspring Weaning	Body mass (g)	36.46	32.28	40.63	36.70	32.53	40.87						
	Lean mass (g)	29.85	26.52	33.18	30.61	27.28	33.95						
	Fat mass (g)	4.014	3.497	4.568	3.746	3.234	4.296						
	Pairing to offspring birth (days)	81.67	56.57	106.8	72.25	46.14	98.36						
	Birth success (%)	0.9442	0.8237	1.065	0.8865	0.7597	1.013						
	Wean success, of all dams (%)	0.7346	0.5340	0.9352	0.8356	0.6273	1.044						
	Wean success, of births (%)	0.7785	0.6058	0.9512	0.9407	0.7573	1.124						
	Litter size	8.857	7.468	10.06	9.025	7.680	10.19						
	Sex ratio (# of females/total)	0.4461	0.3714	0.5208	0.4877	0.4138	0.5616						

Table 3.1. Continued.

	Variable	High Runner											
		Female						Male					
		No Wheel			Wheel			No Wheel			Wheel		
		LSM	LL	UL	LSM	LL	UL	LSM	LL	UL	LSM	LL	UL
Weaning	Body mass (g)	11.52	9.513	13.52				11.76	9.758	13.76			
Wheel Testing	Body mass before wheels (g)	23.18	20.74	25.63				28.91	26.46	31.36			
	Distance (revs/day)	15796	14335	17257				13288	11823	14753			
	Duration (min)	618.3	536.1	700.4				559.4	477.2	641.6			
	Avg. speed (revs/min)	25.80	21.75	30.03				23.70	19.73	27.84			
	Max. speed (revs/min)	40.91	36.09	45.88				38.42	33.67	43.31			
	Body mass after wheels (g)	23.56	20.89	26.23				28.90	26.22	31.57			
Maternal Wheel Access	Body mass before wheels (g)				24.68	22.19	27.16						
	Distance, before breeding (revs/day)				15927	13455	18399						
	Duration, before breeding (min)				553.2	491.6	614.8						
	Avg. speed, before breeding (revs/min)				28.87	22.69	35.04						
	Distance, dams + sires (revs/day)				13091	10708	15474						
	Duration, dams + sires (min)				665.3	561.4	769.2						
	Avg. speed, dams + sires (revs/min)				19.33	16.04	22.77						
	Distance, during pregnancy (revs/day)				5318	4142	6494						
	Duration, during pregnancy (min)				382.6	288.2	476.9						
	Avg. speed, during pregnancy (revs/min)				12.80	10.70	14.89						
	Distance, post-partum (revs/day)				1374	949.7	1927						
	Duration, post-partum (min)				139.1	107.7	174.6						
	Avg. speed, post-partum (revs/min)				10.92	9.275	12.63						
	Body mass after wheels (g)	39.89	35.60	44.18	38.63	34.18	43.08						
Maternal Behavior	Maternal care, days 1-5	0.6217	0.5338	0.7096	0.5218	0.4244	0.6192						
	Maternal care, days 6-10	0.4955	0.3974	0.5936	0.4201	0.3120	0.5282						
	Maternal care, days 11-15	0.4746	0.4062	0.5430	0.4715	0.3878	0.5552						
	Maternal care, days 16-20	0.4103	0.3353	0.4853	0.4188	0.3333	0.5043						
	Maternal care, all days	0.5015	0.4346	0.5684	0.4578	0.3852	0.5304						
	Maternal care, mornings	0.4596	0.3291	0.5901	0.4256	0.2847	0.5665						
	Maternal care, afternoons	0.8114	0.7384	0.8844	0.7761	0.6963	0.8559						
	Maternal care, evenings	0.2578	0.1993	0.3163	0.1883	0.1191	0.2575						
	Nursing, days 1-5	0.5669	0.4843	0.6495	0.4855	0.3915	0.5795						
	Nursing, days 6-10	0.4820	0.3815	0.5825	0.4004	0.2915	0.5093						
	Nursing, days 11-15	0.4534	0.3746	0.5322	0.4502	0.3578	0.5426						
	Nursing, days 16-20	0.2965	0.2111	0.3819	0.3134	0.2197	0.4071						
	Nursing, all days	0.4498	0.3852	0.5144	0.4133	0.3438	0.4828						
	Nursing, mornings	0.4089	0.2920	0.5348	0.3651	0.2434	0.4981						
	Nursing, afternoons	0.7258	0.6501	0.7991	0.7024	0.6195	0.7822						
	Nursing, evenings	0.2288	0.1745	0.2831	0.1796	0.1140	0.2452						
	Activity, days 1-5	0.2783	0.1995	0.3571	0.3569	0.2681	0.4457						
	Activity, days 6-10	0.3092	0.2321	0.3914	0.3954	0.3013	0.4955						
	Activity, all days	0.3397	0.2701	0.4093	0.3913	0.3172	0.4654						
	Activity, mornings	0.3470	0.2289	0.4651	0.4198	0.2926	0.5470						
	Activity, afternoons	0.0892	0.0547	0.1275	0.0984	0.0568	0.1450						
	Activity, evenings	0.5542	0.4704	0.6380	0.6378	0.5417	0.7339						
	Non-wheel activity, days 1-5	0.2746	0.1993	0.3499	0.1596	0.0752	0.2440						
	Non-wheel activity, days 6-10	0.3042	0.2303	0.3829	0.2403	0.1624	0.3251						
	Non-wheel activity, days 11-15	0.3007	0.2082	0.3932	0.3541	0.2533	0.4549						
	Non-wheel activity, days 16-20	0.4578	0.3458	0.5698	0.4579	0.3384	0.5774						
	Non-wheel activity, all days	0.3378	0.2713	0.4043	0.3031	0.2327	0.3735						
	Non-wheel activity, mornings	0.3435	0.2317	0.4553	0.3139	0.1946	0.4332						
	Non-wheel activity, afternoons	0.0893	0.0554	0.1268	0.0887	0.0492	0.1334						
	Non-wheel activity, evenings	0.5515	0.4701	0.6329	0.4898	0.3986	0.5810						
	Maintenance, days 1-5	0.0858	0.0485	0.1279	0.0950	0.0465	0.1509						
	Maintenance, days 6-10	0.1702	0.1131	0.2326	0.1624	0.0966	0.2357						
	Maintenance, days 11-15	0.2087	0.1349	0.2898	0.1635	0.0857	0.2521						
	Maintenance, days 16-20	0.2320	0.1541	0.3099	0.2259	0.1365	0.3153						
	Maintenance, all days	0.1539	0.1089	0.2025	0.1418	0.0941	0.1939						
	Maintenance, mornings	0.1891	0.1313	0.2469	0.1536	0.0864	0.2208						
	Maintenance, afternoons	0.0777	0.0248	0.1424	0.0998	0.0380	0.1738						
	Maintenance, evenings	0.1888	0.1320	0.2456	0.1785	0.1121	0.2449						
	Offspring first eyes open	14.43	13.75	15.10	15.02	14.27	15.76						
	Offspring first movement	13.80	12.69	14.81	13.98	12.68	15.15						
	Offspring first solid food consumption	16.71	16.10	17.32	17.23	16.41	18.06						
Offspring Weaning	Body mass (g)	34.31	30.17	38.45	33.59	29.39	37.79						
	Lean mass (g)	28.08	24.76	31.39	27.58	24.23	30.93						
	Fat mass (g)	3.859	3.369	4.381	3.623	3.096	4.191						
	Pairing to offspring birth (days)	75.17	50.85	99.50	89.69	63.25	116.1						
	Birth success (%)	0.9165	0.7943	1.039	0.8453	0.7085	0.9821						
	Wean success, of all dams (%)	0.8227	0.6544	0.9910	0.6360	0.4468	0.8252						
	Wean success, of births (%)	0.8977	0.7663	1.029	0.7499	0.5912	0.9086						
	Litter size	9.861	8.805	10.81	9.960	8.581	11.17						
	Sex ratio (# of females/total)	0.4475	0.3822	0.5128	0.5906	0.5072	0.6740						

Table 3.2. Least Squares Means (LSMs), F-statistics, and p-values from analyses conducted on the offspring generation. See Table 1 legend for further details.

Table 3.2. Continued.

	Variable	Linetype		Sex		Wheel Access		Linetype* Wheel		Sex*Wheel Access		Linetype* Sex		Linetype* Sex*Wheel	
		F	P	F	P	F	P	F	P	F	P	F	P	F	P
Weaning	Body mass (g)	2.24	0.1849	50.80	**0.0004**	9.53	**0.0215**	0.28	0.6174	0.18	0.6855	6.19	**0.0473**	0.60	0.4666
	Lean mass (g)	4.15	0.0877	50.58	**0.0004**	4.41	0.0804	0.31	0.5975	0.81	0.4039	7.28	**0.0357**	0.34	0.5813
	Fat mass (g)	0.12	0.7431	1.30	0.2980	0.93	0.3713	0.00	0.9806	0.48	0.5144	0.01	0.9191	1.34	0.2904
Home-Cage Activity	Body mass before HCA (g)	4.68	0.0738	338.80	< 0.0001	0.72	0.4284	0.55	0.4855	2.77	0.1471	0.16	0.7001	0.10	0.7666
	Total activity	23.27	**0.0029**	10.05	**0.0193**	1.79	0.2290	2.04	0.2028	0.01	0.9128	4.58	**0.0763**	0.03	0.8713
	Active period per min (activity/min)	11.63	**0.0143**	7.14	**0.0369**	1.63	0.2495	0.61	0.4629	0.00	0.9931	3.94	**0.0944**	0.04	0.8564
	Active periods (min)	11.08	**0.0158**	2.41	0.1718	0.00	0.9882	1.97	0.2101	0.00	0.9693	0.48	0.5142	0.41	0.5476
	Body mass after HCA	5.25	0.0618	229.95	**0.0000**	0.64	0.4554	1.25	0.3068	0.05	0.8355	0.02	0.8997	0.03	0.8689
Wheel Testing	Body mass before wheels (g)	4.00	0.0925	280.53	**< 0.0001**	2.71	0.1509	3.88	**0.0965**	0.84	0.3952	1.55	0.2590	0.00	0.9561
	Lean mass before wheels (g)	2.83	0.1433	492.06	**< 0.0001**	0.88	0.3845	4.68	**0.0738**	0.53	0.4924	3.37	0.1159	0.52	0.4989
	Fat mass before wheels (g)	1.67	0.2443	0.39	0.5560	1.67	0.2439	1.94	0.2133	0.00	0.9943	0.07	0.8038	0.47	0.5203
	Distance (days 5+6) (revs/day)	79.96	**< 0.0001**	11.24	**0.0154**	0.61	0.4629	0.50	0.5074	4.93	**0.0683**	2.45	0.1689	3.02	0.1331
	Duration (days 5+6) (min)	8.78	**0.0252**	32.57	**0.0013**	1.15	0.3239	0.55	0.4874	4.00	**0.0926**	0.08	0.7900	0.82	0.4008
	Avg. speed (days 5+6) (revs/min)	138.26	**< 0.0001**	7.48	**0.0339**	0.00	0.9992	0.01	0.9433	0.86	0.3905	3.95	**0.0940**	0.34	0.5821
	Max. speed (days 5+6) (revs/min)	230.61	**< 0.0001**	5.52	0.0571	0.01	0.9259	0.03	0.8754	1.03	0.3485	2.68	0.1528	0.08	0.7909
	Food consumption (g)	23.09	**0.0030**	1.03	0.3503	0.00	0.9547	0.34	0.5786	0.57	0.4797	2.52	0.1638	1.60	0.2533
	Body mass after wheels (g)	4.08	0.0898	205.81	**< 0.0001**	0.25	0.6359	2.76	0.1476	7.32	**0.0353**	4.40	**0.0807**	0.64	0.4554
	Lean mass after wheels (g)	6.40	**0.0447**	306.68	**< 0.0001**	0.02	0.8855	2.34	0.1772	9.39	**0.0221**	3.05	0.1316	0.03	0.8628
	Fat mass after wheels (g)	0.00	0.9851	1.95	0.2123	0.09	0.7758	0.03	0.8629	0.60	0.4663	8.43	**0.0272**	0.05	0.8234
Open-Field Behavior	Urine and fecal deposits	0.00	0.9856	6.41	**0.0446**	1.62	0.2499	1.33	0.2923	2.03	0.2044	0.34	0.5809	7.01	**0.0381**
	Defacation only	0.01	0.9128	1.85	0.2229	0.55	0.4874	1.12	0.3300	0.00	0.9561	0.47	0.5199	9.23	**0.0229**
	Urination only	0.02	0.8898	7.08	**0.0375**	2.19	0.1891	1.36	0.2874	11.58	**0.0145**	0.45	0.5280	1.14	0.3262
	Latency to leave center (s)	1.19	0.3171	1.01	0.3541	2.15	0.1929	0.41	0.5466	4.30	**0.0836**	0.23	0.6509	0.02	0.9034
	Latency to enter walls (s)	1.25	0.3071	1.85	0.2227	0.76	0.4180	0.58	0.4750	3.35	0.1170	0.12	0.7403	0.00	0.9606
	Duration, walls, 1st min (%)	2.19	0.1893	0.05	0.8388	0.07	0.7946	0.01	0.9388	2.47	0.1674	2.13	0.1952	0.02	0.8956
	Duration, walls, 2nd min (%)	4.60	0.0757	2.23	0.1862	0.16	0.7049	2.39	0.1727	0.59	0.4699	6.59	**0.0425**	0.70	0.4336
	Duration, walls, 3rd min (%)	6.13	**0.0480**	5.23	0.0623	0.18	0.6832	3.04	0.1319	5.00	**0.0668**	3.20	0.1241	0.43	0.5375
	Duration, walls, 4th min (%)	2.40	0.1726	1.96	0.2111	0.50	0.5060	1.46	0.2725	0.14	0.7258	2.00	0.2069	0.00	0.9926
	Duration, walls, 5th min (%)	1.60	0.2525	0.45	0.5259	0.16	0.7064	3.25	0.1214	0.00	0.9493	1.50	0.2668	0.10	0.7598
	Duration, walls, total (%)	3.67	0.1040	1.77	0.2319	0.30	0.6044	2.77	0.1468	0.00	0.9611	4.68	**0.0737**	0.15	0.7114
	Distance, walls, 1st min (cm)	1.79	0.2299	0.03	0.8579	0.81	0.4022	3.31	0.1186	1.07	0.3413	3.05	0.1315	0.00	0.9896
	Distance, walls, 2nd min (cm)	0.25	0.6326	0.59	0.4728	0.43	0.5370	2.30	0.1802	0.05	0.8240	6.01	**0.0497**	0.17	0.6974
	Distance, walls, 3rd min (cm)	1.70	0.2405	0.59	0.4714	0.13	0.7325	0.42	0.5424	0.19	0.6808	2.67	0.1536	0.04	0.8407
	Distance, walls, 4th min (cm)	3.11	0.1284	0.38	0.5592	0.07	0.7975	1.64	0.2474	0.31	0.5970	0.35	0.5749	0.15	0.7114
	Distance, walls, 5th min (cm)	3.25	0.1215	2.61	0.1574	0.01	0.9184	1.02	0.3510	0.14	0.7224	3.18	0.1248	0.81	0.4015
	Distance, walls, total (cm)	0.98	0.3605	1.02	0.3522	0.13	0.7328	1.74	0.2355	0.01	0.9382	4.24	**0.0853**	0.03	0.8758
	Avg. speed, walls, 1st min (cm/s)	7.09	**0.0374**	0.08	0.7842	1.04	0.3467	4.99	**0.0670**	0.22	0.6540	8.91	**0.0245**	0.44	0.5324
	Avg. speed, walls, 2nd min (cm/s)	0.92	0.3752	0.33	0.5857	0.41	0.5476	2.15	0.1925	0.01	0.9088	7.30	**0.0355**	0.01	0.9254
	Avg. speed, walls, 3rd min (cm/s)	0.03	0.8736	0.07	0.7976	1.02	0.3507	0.72	0.4297	0.64	0.4557	3.25	0.1213	0.30	0.6013
	Avg. speed, walls, 4th min (cm/s)	0.21	0.6617	0.42	0.5405	0.18	0.6832	1.56	0.2582	0.99	0.3578	0.42	0.5411	0.18	0.6836
	Avg. speed, walls, 5th min (cm/s)	0.12	0.7432	2.32	0.1782	0.25	0.6380	3.95	**0.0940**	0.03	0.8674	1.77	0.2315	0.84	0.3944
	Avg. speed, walls, total (cm/s)	0.26	0.6302	0.17	0.6944	0.48	0.5153	2.55	0.1616	0.52	0.4971	4.24	**0.0853**	0.01	0.9374
	Distance, all, 1st min (cm)	4.47	0.0790	1.41	0.2803	0.82	0.4010	3.07	0.1301	0.22	0.6575	6.02	**0.0496**	0.04	0.8485
	Distance, all, 2nd min (cm)	1.04	0.3477	0.12	0.7432	0.77	0.4146	1.25	0.3054	0.00	0.9876	4.94	**0.0679**	0.12	0.7369
	Distance, all, 3rd min (cm)	0.62	0.4608	0.21	0.6647	0.54	0.4896	1.36	0.2880	1.92	0.2147	4.05	**0.0907**	0.11	0.7499
	Distance, all, 4th min (cm)	0.01	0.9258	0.09	0.7689	0.20	0.6666	1.47	0.2702	1.34	0.2907	0.78	0.4111	0.33	0.5852
	Distance, all, 5th min (cm)	0.03	0.8750	1.52	0.2640	0.26	0.6297	3.20	0.1237	0.02	0.8951	1.69	0.2407	0.12	0.7367
	Distance, all, total (cm)	0.58	0.4753	0.00	0.9815	0.58	0.4749	2.23	0.1864	0.52	0.4994	4.21	**0.0859**	0.00	0.9481
	Avg. speed, all, 1st min (cm/s)	5.40	0.0591	1.49	0.2680	0.82	0.3991	3.18	0.1246	0.22	0.6563	6.59	**0.0425**	0.04	0.8488
	Avg. speed, all, 2nd min (cm/s)	1.32	0.2937	0.15	0.7134	1.17	0.3207	1.87	0.2200	0.00	0.9467	6.64	**0.0419**	0.16	0.7028
	Avg. speed, all, 3rd min (cm/s)	0.62	0.4609	0.21	0.6629	0.54	0.4893	1.37	0.2855	1.94	0.2131	4.06	**0.0905**	0.11	0.7478
	Avg. speed, all, 4th min (cm/s)	0.01	0.9274	0.10	0.7652	0.20	0.6688	1.49	0.2685	1.32	0.2938	0.79	0.4096	0.32	0.5902
	Avg. speed, all, 5th min (cm/s)	6.03	**0.0495**	1.34	0.2907	0.64	0.4530	3.54	0.1090	0.17	0.6923	5.89	**0.0514**	0.03	0.8749
	Avg. speed, all, total (cm/s)	2.01	0.2058	5.31	0.0608	2.08	0.1997	2.13	0.1950	1.09	0.3376	1.46	0.2720	0.01	0.9150
Offspring Weaning	Body mass (g)	2.31	0.1797			0.06	0.8105	2.73	0.1496						
	Lean mass (g)	3.11	0.1283			0.51	0.5037	1.88	0.2198						
	Fat mass (g)	0.28	0.6145			0.21	0.6619	0.08	0.7925						
	Pairing to offspring birth (days)	1.79	0.2298			0.07	0.8056	0.11	0.7462						
	Birth success (%)	5.59	0.0560			2.45	0.1689	0.64	0.4533						
	Wean success, of all dams (%)	2.65	0.1548			1.16	0.3237	0.27	0.6230						
	Wean success, of births (%)	0.01	0.9332			0.03	0.8773	0.20	0.6738						
	Litter size	0.00	0.9737			0.00	0.9808	0.03	0.8621						
	Sex ratio (# of females/total)	0.78	0.4123			2.42	0.1708	0.17	0.6961						
Dissec-tion	Body mass (g)	6.03	**0.0494**	7.18	**0.0366**	0.20	0.6730	3.43	0.1133	0.07	0.7953	0.15	0.7157	0.06	0.8101
	Lean mass (g)	3.73	0.1016	5.31	0.0606	0.33	0.5862	3.62	0.1057	0.37	0.5638	0.03	0.8603	0.24	0.6427
	Fat mass (g)	0.82	0.3999	0.39	0.5545	1.73	0.2362	1.75	0.2336	2.58	0.1594	0.06	0.8215	0.92	0.3752
	Heart ventricle (g)	1.39	0.2833	0.01	0.9105	0.15	0.7145	0.47	0.5172	0.24	0.6440	2.78	0.1467	0.91	0.3766
	Liver (g)	0.07	0.7985	3.13	0.1273	0.30	0.6053	0.08	0.7882	0.01	0.9370	0.51	0.5026	0.32	0.5907
	Triceps surae (g)	0.30	0.6010	0.20	0.6730	1.50	0.2665	0.23	0.6469	0.06	0.8093	4.68	**0.0736**	1.54	0.2609
	Kidney, avg. (g)	0.04	0.8501	37.11	**0.0009**	0.16	0.7016	0.00	0.9956	0.05	0.8302	0.02	0.8824	1.65	0.2467
	Spleen (g)	0.00	0.9870	4.38	0.0812	0.17	0.6948	0.09	0.7690	0.41	0.5453	0.28	0.6187	3.41	0.1142
	Reproductive fat pads (g)	0.02	0.8798	23.46	**0.0029**	0.85	0.3917	0.07	0.8020	0.01	0.9232	2.82	0.1441	1.68	0.2420
	Subdermal fat pads (g)	0.09	0.7735	1.12	0.3314	1.17	0.3219	0.07	0.8029	5.22	**0.0624**	0.78	0.4100	0.53	0.4941
	Whole-brain (g)	8.02	**0.0299**	0.33	0.5864	0.01	0.9093	0.07	0.8022	0.91	0.3773	0.36	0.5695	0.00	0.9639
	Cerebrum (g)	8.03	**0.0298**	1.29	0.3000	0.02	0.8921	0.12	0.7430	0.38	0.5589	0.55	0.4859	0.28	0.6177
	Cerebellum (g)	3.01	0.1334	0.44	0.5340	0.43	0.5369	0.20	0.6736	1.69	0.2411	0.80	0.4069	0.10	0.7576
	Anterior-posterior length (cm)	1.78	0.2300	1.07	0.3410	1.28	0.3009	1.94	0.2135	0.13	0.7300	0.63	0.4563	0.01	0.9332
	Cerebrum area (cm)	0.71	0.4318	10.63	**0.0172**	3.50	0.1107	4.55	**0.0768**	0.17	0.6913	2.51	0.1640	0.00	0.9721
	Cerebellum area (cm)	0.39	0.5547	6.90	**0.0392**	0.94	0.3686	0.01	0.9084	0.09	0.7767	0.87	0.3869	0.10	0.7674

Table 3.2. Continued.

	Variable	Control											
		Female						Male					
		No Wheel			Wheel			No Wheel			Wheel		
		LSM	LL	UL	LSM	LL	UL	LSM	LL	UL	LSM	LL	UL
Weaning	Body mass (g)	13.48	11.95	14.93	12.77	11.14	14.30	14.21	12.72	15.63	13.35	11.76	14.85
	Lean mass (g)	11.05	9.844	12.26	10.76	9.526	12.00	11.74	10.53	12.95	11.27	10.03	12.51
	Fat mass (g)	1.348	0.9750	1.722	1.318	0.9388	1.696	1.396	1.022	1.770	1.307	0.9266	1.687
Home-Cage Activity	Body mass before HCA (g)	21.86	19.98	23.74	22.24	20.39	24.10	26.77	24.89	28.65	26.26	24.39	28.13
	Total activity	207.5	155.3	259.6	205.9	157.9	253.9	192.6	143.3	241.9	189.8	140.8	238.9
	Active period per min (activity/min)	0.3791	0.3115	0.4467	0.3631	0.3002	0.4260	0.3642	0.2998	0.4286	0.3550	0.2909	0.4191
	Active periods (min)	543.2	461.8	624.6	572.9	497.9	647.9	529.2	452.3	606.1	541.6	465.0	618.2
	Body mass after HCA	22.57	20.23	24.91	22.78	20.50	25.06	27.10	24.79	29.40	27.29	24.99	29.59
Wheel Testing	Body mass before wheels (g)	24.66	22.32	27.01	24.88	22.54	27.22	31.18	28.84	33.53	31.15	28.80	33.50
	Lean mass before wheels (g)	20.03	18.23	21.83	20.61	18.81	22.40	26.24	24.44	28.03	26.29	24.50	28.09
	Fat mass before wheels (g)	2.695	1.939	3.575	2.646	1.906	3.508	2.769	1.994	3.670	2.843	2.056	3.757
	Distance (revs/day)	3820	2632	5007	3650	2457	4843	2581	1391	3771	2686	1489	3883
	Duration (min)	403.7	320.7	486.8	383.1	299.7	466.4	283.3	200.0	366.5	292.8	209.2	376.4
	Avg. speed (revs/min)	9.371	7.464	11.28	9.172	7.255	11.09	8.808	6.894	10.72	8.906	6.979	10.83
	Max. speed (revs/min)	18.13	15.92	20.49	17.94	15.73	20.30	17.36	15.19	19.68	17.89	15.66	20.26
	Food consumption (g)	30.70	28.23	33.16	31.11	28.74	33.48	31.38	28.78	33.97	30.36	27.79	32.92
	Body mass after wheels (g)	24.86	22.39	27.33	25.63	23.17	28.09	32.10	29.63	34.56	31.95	29.48	34.41
	Lean mass after wheels (g)	20.86	18.98	22.74	21.51	19.64	23.39	27.56	25.68	29.43	27.47	25.60	29.35
	Fat mass after wheels (g)	1.595	1.150	2.040	1.661	1.219	2.102	1.687	1.231	2.143	1.675	1.220	2.129
Open-Field Behavior	Urine and fecal deposits	3.610	1.920	5.533	3.006	1.414	4.851	3.947	2.177	5.947	4.465	2.631	6.514
	Defacation only	2.294	1.051	3.741	1.417	0.3701	2.728	1.506	0.4130	2.868	2.685	1.349	4.218
	Urination only	0.8913	0.1687	1.827	1.282	0.4627	2.269	2.178	1.187	3.301	1.563	0.6718	2.610
	Latency to leave center (s)	11.31	6.982	16.68	11.24	7.000	16.48	8.231	4.604	12.90	13.36	8.612	19.15
	Latency to enter walls (s)	14.71	9.193	21.53	15.44	9.859	22.26	9.923	5.493	15.65	15.55	9.765	22.67
	Duration, walls, 1st min (%)	61.78	48.75	72.49	65.47	53.18	75.79	61.87	48.85	72.59	56.79	42.31	68.26
	Duration, walls, 2nd min (%)	82.86	67.22	95.47	83.82	68.40	96.31	72.08	52.21	86.72	79.16	62.26	92.43
	Duration, walls, 3rd min (%)	88.48	76.51	97.88	87.25	74.88	96.85	69.00	43.73	83.07	81.42	66.61	92.21
	Duration, walls, 4th min (%)	82.33	64.48	96.95	87.67	71.10	101.6	70.71	48.68	87.34	79.55	60.82	94.63
	Duration, walls, 5th min (%)	79.30	59.30	95.19	86.98	69.09	101.8	70.96	47.33	88.49	82.07	62.85	97.57
	Duration, walls, total (%)	77.62	63.47	89.11	81.34	68.12	92.30	67.73	49.94	81.02	73.90	58.60	86.00
	Distance, walls, 1st min (cm)	3313	2271	4446	2684	1706	3763	4162	3047	5358	2928	1900	4057
	Distance, walls, 2nd min (cm)	4932	3351	6653	3730	2275	5347	5278	3647	7048	4460	2900	6173
	Distance, walls, 3rd min (cm)	4608	2995	6222	4147	2535	5759	4886	3255	6517	4555	2921	6190
	Distance, walls, 4th min (cm)	4627	3231	6137	4338	2969	5825	4665	3249	6199	4281	2899	5785
	Distance, walls, 5th min (cm)	4356	3205	5590	4006	2884	5215	4542	3338	5832	3913	2779	5136
	Distance, walls, total (cm)	22038	15562	28514	19169	12696	25642	23437	16903	29971	20391	13824	26958
	Avg. speed, walls, 1st min (cm/s)	98.16	73.09	125.0	73.79	50.68	98.88	115.3	89.03	143.2	91.86	66.82	118.8
	Avg. speed, walls, 2nd min (cm/s)	109.1	72.55	149.0	89.51	55.06	127.7	125.1	86.76	166.6	101.9	65.64	141.8
	Avg. speed, walls, 3rd min (cm/s)	100.6	67.74	136.4	95.75	63.40	131.1	121.3	86.18	159.2	97.03	64.14	133.0
	Avg. speed, walls, 4th min (cm/s)	108.0	74.36	144.6	97.39	64.74	133.1	111.9	77.48	149.2	93.65	60.91	129.6
	Avg. speed, walls, 5th min (cm/s)	104.3	73.21	139.0	89.11	59.87	122.1	108.1	75.63	144.2	83.16	54.29	116.0
	Avg. speed, walls, total (cm/s)	101.8	73.41	134.9	88.94	62.56	119.9	116.1	85.30	151.7	93.43	65.97	125.7
	Distance, all, 1st min (cm)	5407	3655	7158	4401	2651	6151	6619	4850	8387	5447	3675	7220
	Distance, all, 2nd min (cm)	7050	4240	9859	5795	2986	8604	8137	5310	10964	6526	3687	9364
	Distance, all, 3rd min (cm)	6296	4270	8501	6054	4054	8236	8026	5832	10383	6351	4297	8588
	Distance, all, 4th min (cm)	6713	4584	9028	6063	3998	8324	7188	4994	9566	6063	3970	8356
	Distance, all, 5th min (cm)	6598	4493	8889	5537	3538	7736	6722	4580	9052	5476	3457	7703
	Distance, all, total (cm)	31177	21992	41961	27304	18767	37437	36405	26322	48119	29467	20459	40114
	Avg. speed, all, 1st min (cm/s)	90.12	63.10	117.1	73.33	46.33	100.3	110.2	82.89	137.5	90.75	63.37	118.1
	Avg. speed, all, 2nd min (cm/s)	117.0	77.43	156.6	96.04	56.47	135.6	135.4	95.44	175.4	107.9	67.82	148.0
	Avg. speed, all, 3rd min (cm/s)	104.8	71.11	141.5	100.8	67.52	137.1	133.7	97.19	172.9	105.7	71.54	142.9
	Avg. speed, all, 4th min (cm/s)	111.8	76.36	150.3	101.0	66.62	138.6	119.7	83.19	159.2	101.0	66.15	139.1
	Avg. speed, all, 5th min (cm/s)	88.46	62.29	116.8	71.69	47.08	98.66	108.9	80.81	138.9	88.53	62.00	117.2
	Avg. speed, all, total (cm/s)	104.4	69.19	146.7	89.42	57.17	128.9	137.7	96.40	186.3	104.5	69.02	147.4
Offspring Weaning	Body mass (g)	37.92	33.85	41.99	38.64	34.58	42.69						
	Lean mass (g)	30.84	28.08	33.60	31.11	28.37	33.85						
	Fat mass (g)	4.217	3.424	5.126	4.333	3.533	5.246						
	Pairing to offspring birth (days)	80.67	52.21	109.1	81.55	53.74	109.4						
	Birth success (%)	0.9198	0.8154	1.024	0.9818	0.8809	1.083						
	Wean success, of all dams (%)	0.8394	0.7083	0.9705	0.9085	0.7818	1.035						
	Wean success, of births (%)	0.9127	0.8138	1.012	0.9250	0.8325	1.017						
	Litter size	10.46	8.766	11.92	10.37	8.697	11.81						
	Sex ratio (# of females/total)	0.4755	0.3954	0.5591	0.4448	0.3713	0.5215						
Dissec-tion	Body mass (g)	35.15	31.63	38.68	35.74	32.19	39.29	38.44	35.03	41.85	39.04	35.67	42.42
	Lean mass (g)	28.65	25.76	31.55	29.16	26.24	32.07	31.35	28.53	34.17	31.49	28.70	34.27
	Fat mass (g)	4.839	3.092	7.150	4.774	3.037	7.076	4.307	2.721	6.417	4.377	2.783	6.488
	Heart ventricle (g)	0.1561	0.1411	0.1726	0.1615	0.1454	0.1795	0.1565	0.1412	0.1734	0.1566	0.1417	0.1732
	Liver (g)	2.479	2.270	2.706	2.463	2.245	2.703	2.150	1.961	2.357	2.111	1.931	2.307
	Triceps surae (g)	0.1109	0.0997	0.1234	0.1121	0.1005	0.1250	0.1082	0.0972	0.1204	0.1138	0.1025	0.1264
	Kidney, avg. (g)	0.4440	0.4062	0.4854	0.4494	0.4087	0.4941	0.6433	0.5871	0.7049	0.6289	0.5753	0.6876
	Spleen (g)	0.1170	0.0867	0.1579	0.1204	0.0888	0.1632	0.0972	0.0719	0.1315	0.0911	0.0676	0.1229
	Reproductive fat pads (g)	0.0887	-0.1232	0.3006	0.0528	-0.1624	0.2680	0.5661	0.3519	0.7803	0.5644	0.3520	0.7768
	Subdermal fat pads (g)	0.3066	0.1977	0.4262	0.2626	0.1547	0.3830	0.2254	0.1233	0.3409	0.2463	0.1435	0.3613
	Whole-brain (g)	0.4789	0.4507	0.5088	0.4827	0.4534	0.5139	0.4716	0.4435	0.5015	0.4710	0.4435	0.5002
	Cerebrum (g)	0.4143	0.3854	0.4397	0.4174	0.3879	0.4433	0.3991	0.3672	0.4265	0.3971	0.3656	0.4242
	Cerebellum (g)	0.0659	0.0583	0.0746	0.0685	0.0602	0.0780	0.0712	0.0626	0.0809	0.0717	0.0633	0.0812
	Anterior-posterior length (cm)	0.6089	0.5642	0.6536	0.6270	0.5799	0.6741	0.5912	0.5469	0.6355	0.6044	0.5611	0.6477
	Cerebrum area (cm)	0.7280	0.6953	0.7607	0.7404	0.7067	0.7741	0.6889	0.6564	0.7214	0.7036	0.6716	0.7356
	Cerebellum area (cm)	0.3492	0.3257	0.3727	0.3445	0.3199	0.3691	0.3169	0.2937	0.3401	0.3158	0.2931	0.3385

Table 3.2. Continued.

	Variable	High Runner											
		Female						Male					
		No Wheel			Wheel			No Wheel			Wheel		
		LSM	LL	UL	LSM	LL	UL	LSM	LL	UL	LSM	LL	UL
Weaning	Body mass (g)	12.63	11.09	14.09	11.44	9.664	13.08	12.93	11.40	14.38	11.80	10.06	13.42
	Lean mass (g)	10.05	8.877	11.22	9.414	8.171	10.66	10.34	9.166	11.51	9.665	8.421	10.91
	Fat mass (g)	1.438	1.072	1.803	1.367	0.9857	1.748	1.452	1.087	1.817	1.397	1.016	1.778
Home-Cage Activity	Body mass before HCA (g)	20.58	18.69	22.47	19.95	17.98	21.92	25.16	23.28	27.04	23.92	21.95	25.89
	Total activity	345.8	296.7	395.0	305.3	251.3	359.4	261.6	211.0	312.1	227.4	174.6	280.2
	Active period per min (activity/min)	0.4971	0.4330	0.5612	0.4569	0.3869	0.5269	0.4203	0.3547	0.4859	0.3739	0.3051	0.4427
	Active periods (min)	690.4	613.6	767.3	660.1	575.9	744.4	621.0	542.0	699.9	610.4	528.5	692.2
	Body mass after HCA	20.61	18.30	22.93	19.50	17.11	21.88	25.31	23.00	27.61	24.00	21.55	26.44
Wheel Testing	Body mass before wheels (g)	23.09	20.76	25.43	22.24	19.86	24.62	28.74	26.41	31.08	27.61	25.23	29.98
	Lean mass before wheels (g)	19.54	17.76	21.33	18.75	16.91	20.59	24.58	22.80	26.37	23.79	21.95	25.62
	Fat mass before wheels (g)	2.270	1.581	3.084	2.069	1.392	2.880	2.396	1.695	3.219	2.083	1.426	2.863
	Distance (revs/day)	12251	9932	14570	10510	8031	12989	8098	5800	10397	8608	6159	11057
	Duration (min)	553.6	458.9	648.4	483.2	385.0	581.4	418.0	323.8	512.3	427.6	330.2	525.0
	Avg. speed (revs/min)	22.37	19.71	25.03	21.76	18.77	24.76	19.09	16.47	21.72	19.79	16.85	22.74
	Max. speed (revs/min)	36.56	33.37	39.90	35.61	32.03	39.37	32.46	29.52	35.55	33.26	29.88	36.81
	Food consumption (g)	37.26	34.77	39.74	37.32	34.63	40.02	35.60	33.22	37.97	36.03	33.59	38.47
	Body mass after wheels (g)	23.58	21.12	26.03	23.25	20.76	25.75	28.87	26.42	31.33	28.05	25.56	30.54
	Lean mass after wheels (g)	19.22	17.35	21.08	19.20	17.30	21.10	24.73	22.86	26.60	24.05	22.15	25.95
	Fat mass after wheels (g)	1.783	1.333	2.234	1.811	1.353	2.269	1.509	1.069	1.949	1.495	1.050	1.940
Open-Field Behavior	Urine and fecal deposits	2.756	1.173	4.619	3.372	1.478	5.590	6.179	4.123	8.424	2.963	1.148	5.129
	Defacation only	1.422	0.3500	2.773	1.705	0.3773	3.398	3.256	1.811	4.886	1.362	0.1514	2.998
	Urination only	1.145	0.3444	2.132	1.435	0.4714	2.610	2.659	1.590	3.850	0.9672	0.1232	2.097
	Latency to leave center (s)	14.95	9.867	21.09	13.45	8.041	20.24	10.53	6.303	15.85	14.07	8.660	20.79
	Latency to enter walls (s)	18.55	12.24	26.17	15.73	9.214	23.98	13.97	8.515	20.76	17.12	10.40	25.50
	Duration, walls, 1st min (%)	66.15	54.33	76.16	68.59	55.86	79.31	72.23	61.48	81.58	68.16	55.63	78.72
	Duration, walls, 2nd min (%)	94.72	81.96	105.6	92.98	79.34	104.5	96.42	83.94	107.2	94.52	81.31	105.7
	Duration, walls, 3rd min (%)	97.18	87.61	105.2	92.71	81.49	101.7	93.69	83.28	102.2	94.07	83.35	102.8
	Duration, walls, 4th min (%)	92.27	76.77	105.5	89.56	72.73	103.7	91.23	75.44	104.7	90.72	74.22	104.6
	Duration, walls, 5th min (%)	90.94	74.21	105.0	86.35	66.98	102.1	93.34	77.00	107.2	87.29	68.22	102.9
	Duration, walls, total (%)	86.75	74.80	96.93	85.23	72.42	96.00	88.99	77.43	98.92	86.41	73.90	96.99
	Distance, walls, 1st min (cm)	2633	1653	3715	3218	1978	4594	2499	1499	3613	2517	1381	3803
	Distance, walls, 2nd min (cm)	5272	3642	7040	5752	3797	7892	4280	2706	6017	4622	2799	6650
	Distance, walls, 3rd min (cm)	6029	4396	7662	5953	4124	7783	4887	3217	6558	5189	3350	7029
	Distance, walls, 4th min (cm)	5481	3996	7076	6322	4625	8143	5278	3775	6898	5537	3895	7310
	Distance, walls, 5th min (cm)	5445	4198	6767	5511	4110	7009	4184	2994	5467	4928	3565	6393
	Distance, walls, total (cm)	25535	19012	32058	26967	19423	34511	21446	14782	28110	23402	15815	30989
	Avg. speed, walls, 1st min (cm/s)	64.90	42.56	89.38	78.91	51.29	109.2	57.33	35.21	81.87	59.78	34.53	88.09
	Avg. speed, walls, 2nd min (cm/s)	95.29	59.91	134.4	103.7	62.15	150.0	74.75	41.21	112.7	82.42	43.82	126.3
	Avg. speed, walls, 3rd min (cm/s)	107.9	73.88	144.9	108.3	71.23	149.0	95.09	61.98	131.4	92.16	56.72	131.4
	Avg. speed, walls, 4th min (cm/s)	106.4	72.42	143.3	123.5	83.96	166.4	107.2	72.84	144.7	104.7	66.89	146.4
	Avg. speed, walls, 5th min (cm/s)	108.3	76.28	143.9	113.4	77.40	153.8	82.46	53.34	115.6	101.4	66.82	140.6
	Avg. speed, walls, total (cm/s)	94.06	66.56	126.3	104.6	72.02	143.2	84.40	58.22	115.4	87.57	57.89	123.4
	Distance, all, 1st min (cm)	3560	1789	5330	4114	2151	6077	3373	1567	5180	3514	1543	5485
	Distance, all, 2nd min (cm)	6100	3277	8924	6112	2948	9277	4697	1827	7568	5034	1857	8212
	Distance, all, 3rd min (cm)	5854	3821	8083	6483	4211	8976	5657	3651	7861	5453	3287	7863
	Distance, all, 4th min (cm)	6296	4182	8606	7417	4958	10101	6514	4358	8867	6219	3873	8813
	Distance, all, 5th min (cm)	6573	4441	8896	7020	4633	9632	5294	3273	7532	6133	3832	8677
	Distance, all, total (cm)	27696	19002	38022	30475	20360	42622	25463	17085	35507	26005	16701	37360
	Avg. speed, all, 1st min (cm/s)	59.33	32.00	86.66	68.53	37.79	99.26	55.97	28.02	83.91	58.56	27.67	89.44
	Avg. speed, all, 2nd min (cm/s)	101.7	61.74	141.6	102.2	57.32	147.1	78.97	38.21	119.7	84.08	38.95	129.2
	Avg. speed, all, 3rd min (cm/s)	97.45	63.64	134.5	108.0	70.18	149.5	94.19	60.82	130.9	90.83	54.80	130.9
	Avg. speed, all, 4th min (cm/s)	104.9	69.70	143.3	123.5	82.58	168.1	108.3	72.47	147.4	103.6	64.56	146.7
	Avg. speed, all, 5th min (cm/s)	56.75	33.48	82.74	66.27	39.02	96.71	53.58	30.21	79.89	57.52	31.31	87.20
	Avg. speed, all, total (cm/s)	76.00	46.23	113.1	81.48	48.26	123.4	87.93	55.39	127.9	82.46	48.93	124.7
Offspring Weaning	Body mass (g)	35.26	31.18	39.34	34.29	30.14	38.44						
	Lean mass (g)	28.65	25.88	31.42	27.80	24.95	30.66						
	Fat mass (g)	4.522	3.680	5.486	4.552	3.659	5.581						
	Pairing to offspring birth (days)	101.6	72.95	130.2	95.30	64.76	125.8						
	Birth success (%)	0.8045	0.6719	0.9371	0.8702	0.7212	1.019						
	Wean success, of all dams (%)	0.7537	0.5934	0.9140	0.7917	0.6101	0.9733						
	Wean success, of births (%)	0.9334	0.8336	1.033	0.9086	0.7917	1.025						
	Litter size	10.33	8.610	11.81	10.45	8.598	12.02						
	Sex ratio (# of females/total)	0.5218	0.4409	0.6059	0.4676	0.3794	0.5602						
Dissec-tion	Body mass (g)	31.61	27.99	35.22	30.51	26.91	34.12	35.12	31.64	38.59	34.28	30.73	37.83
	Lean mass (g)	26.49	23.49	29.48	25.92	22.94	28.90	28.88	26.07	31.68	28.27	25.40	31.14
	Fat mass (g)	4.175	2.583	6.318	3.612	2.179	5.569	3.627	2.259	5.461	3.547	2.178	5.400
	Heart ventricle (g)	0.1632	0.1475	0.1804	0.1614	0.1457	0.1789	0.1669	0.1515	0.1839	0.1669	0.1505	0.1851
	Liver (g)	2.449	2.111	2.842	2.420	2.084	2.811	2.205	1.907	2.551	2.215	1.906	2.573
	Triceps surae (g)	0.1089	0.0986	0.1203	0.1118	0.1010	0.1239	0.1166	0.1059	0.1283	0.1165	0.1052	0.1290
	Kidney, avg. (g)	0.4458	0.3978	0.4995	0.4381	0.3902	0.4919	0.6317	0.5654	0.7058	0.6361	0.5667	0.7139
	Spleen (g)	0.1187	0.0879	0.1604	0.1158	0.0856	0.1566	0.0939	0.0697	0.1264	0.0958	0.0706	0.1299
	Reproductive fat pads (g)	0.1441	-0.0134	0.3017	0.1484	-0.01	0.3069	0.4766	0.3210	0.6322	0.4516	0.2906	0.6126
	Subdermal fat pads (g)	0.3280	0.2167	0.4497	0.2920	0.1837	0.4115	0.2405	0.1406	0.3522	0.2393	0.1353	0.3562
	Whole-brain (g)	0.5170	0.4860	0.5500	0.5186	0.4874	0.5517	0.5125	0.4824	0.5445	0.5096	0.4784	0.5428
	Cerebrum (g)	0.4462	0.4210	0.4687	0.4450	0.4198	0.4676	0.4345	0.4086	0.4576	0.4329	0.4053	0.4573
	Cerebellum (g)	0.0733	0.0645	0.0833	0.0755	0.0661	0.0861	0.0774	0.0683	0.0877	0.0758	0.0665	0.0863
	Anterior-posterior length (cm)	0.6429	0.6022	0.6836	0.6427	0.6020	0.6834	0.6110	0.5708	0.6512	0.6077	0.5649	0.6505
	Cerebrum area (cm)	0.7556	0.7342	0.7770	0.7532	0.7317	0.7747	0.6918	0.6706	0.7130	0.6922	0.6698	0.7146
	Cerebellum area (cm)	0.3461	0.3223	0.3699	0.3424	0.3187	0.3661	0.3096	0.2861	0.3331	0.3058	0.2812	0.3304

Table 3.3. Least Squares Means (LSMs), F-statistics, and p-values from analyses conducted on the grand-offspring generation. See Table 1 legend for further details.

	Variable	Linetype		Sex		Wheel Access		Linetype* Wheel		Sex*Wheel Access		Linetype* Sex		Linetype* Sex*Wheel	
		F	P	F	P	F	P	F	P	F	P	F	P	F	P
Weaning	Body mass (g)	0.01	0.9429	17.60	**0.0057**	0.02	0.8928	0.74	0.4231	0.03	0.8594	0.05	0.8228	0.04	0.8558
	Lean mass (g)	0.15	0.7113	26.49	**0.0021**	0.13	0.7299	0.98	0.3602	0.27	0.6210	0.28	0.6164	0.61	0.4659
	Fat mass (g)	3.15	0.1265	0.39	0.5547	0.08	0.7833	0.00	0.9626	0.32	0.5929	0.00	0.9967	1.29	0.2987
Wheel Testing	Body mass before wheels (g)	4.47	0.0790	362.32	**< 0.0001**	0.51	0.5031	3.27	0.1203	0.04	0.8577	5.77	**0.0532**	2.43	0.1703
	Lean mass before wheels (g)	3.75	0.1010	333.52	**< 0.0001**	0.00	0.9912	1.08	0.3386	0.50	0.5080	6.14	**0.0480**	2.52	0.1636
	Fat mass before wheels (g)	1.30	0.2975	0.00	0.9478	1.15	0.3247	0.35	0.5762	4.08	**0.0900**	2.44	0.1693	1.15	0.3241
	Distance (revs/day)	107.95	**< 0.0001**	5.72	0.0539	0.24	0.6428	0.16	0.6999	2.07	0.2002	0.12	0.7437	1.01	0.3543
	Duration (min)	6.58	**0.0426**	32.78	**0.0012**	0.14	0.7179	0.08	0.7864	0.13	0.7303	7.95	**0.0304**	0.49	0.5083
	Avg. speed (revs/min)	85.60	**< 0.0001**	1.66	0.2456	0.65	0.4514	0.33	0.5849	2.78	0.1462	0.73	0.4245	0.34	0.5822
	Max. speed (revs/min)	102.58	**< 0.0001**	2.37	0.1746	0.08	0.7822	0.65	0.4512	3.34	0.1173	0.04	0.8534	0.26	0.6270
	Food consumption (g)	18.69	**0.0050**	0.02	0.8992	1.69	0.2411	0.22	0.6556	6.03	**0.0494**	3.17	0.1255	0.27	0.6242
	Body mass after wheels (g)	2.28	0.1814	345.03	**< 0.0001**	0.01	0.9284	4.82	**0.0705**	0.39	0.5572	11.70	**0.0141**	7.50	**0.0338**
	Lean mass after wheels (g)	3.66	0.1044	298.80	**< 0.0001**	0.01	0.9447	2.96	0.1362	0.13	0.7268	7.06	**0.0376**	3.27	0.1206
	Fat mass after wheels (g)	0.01	0.9084	3.41	0.1145	0.01	0.9102	1.39	0.2829	0.14	0.7214	3.65	0.1045	0.00	0.9527
Offspring Weaning	Body mass (g)	0.48	0.5150			0.00	0.9963	0.04	0.8501						
	Pairing to offspring birth (days)	1.81	0.2272			0.07	0.7980	2.22	0.1866						
	Birth success (%)	GLIMMIX did not converge													
	Wean success, of all dams (%)	1.59	0.2547			1.48	0.2692	0.24	0.6444						
	Wean success, of births (%)	1.01	0.3534			0.83	0.3969	0.01	0.9354						
	Litter size	1.61	0.2514			3.25	0.1217	1.30	0.2978						
	Sex ratio (# of females/total)	0.02	0.8960			0.01	0.9360	0.24	0.6393						

	Variable	Control											
		Female						Male					
		No Wheel			Wheel			No Wheel			Wheel		
		LSM	LL	UL	LSM	LL	UL	LSM	LL	UL	LSM	LL	UL
Weaning	Body mass (g)	12.48	10.25	14.37	12.80	10.62	14.67	12.96	10.83	14.79	13.28	11.18	15.08
	Lean mass (g)	10.53	8.966	11.89	10.65	9.090	12.01	10.89	9.384	12.21	11.18	9.702	12.48
	Fat mass (g)	1.168	0.8826	1.454	1.211	0.9199	1.503	1.205	0.9191	1.491	1.204	0.9129	1.496
Wheel Testing	Body mass before wheels (g)	25.15	23.04	27.27	25.26	23.15	27.37	31.59	29.48	33.70	32.17	30.06	34.28
	Lean mass before wheels (g)	20.65	18.95	22.34	20.49	18.80	22.19	26.53	24.83	28.22	27.26	25.57	28.95
	Fat mass before wheels (g)	2.879	2.146	3.661	3.016	2.273	3.807	2.959	2.204	3.765	2.722	1.979	3.521
	Distance (revs/day)	4519	2946	6091	4657	3068	6246	3320	1759	4880	3243	1698	4788
	Duration (min)	471.2	361.1	581.2	457.7	346.7	568.7	332.6	222.9	442.4	326.2	217.2	435.2
	Avg. speed (revs/min)	9.212	7.205	11.22	9.737	7.702	11.77	9.351	7.365	11.34	9.080	7.125	11.04
	Max. speed (revs/min)	19.70	16.88	22.51	20.01	17.15	22.87	19.38	16.60	22.17	18.38	15.64	21.11
	Food consumption (g)	32.31	29.26	35.37	31.44	28.24	34.64	31.85	28.41	35.28	34.02	30.47	37.57
	Body mass after wheels (g)	24.27	22.06	26.48	24.36	22.14	26.58	30.64	28.42	32.85	31.74	29.53	33.95
	Lean mass after wheels (g)	20.65	18.79	22.51	20.74	18.87	22.61	26.92	25.07	28.77	27.62	25.76	29.48
	Fat mass after wheels (g)	1.867	1.232	2.561	1.928	1.283	2.633	1.771	1.133	2.473	1.861	1.206	2.581
Offspring Weaning	Body mass (g)	36.54	32.45	40.64	36.68	32.56	40.80						
	Pairing to offspring birth (days)	47.34	32.55	62.13	54.66	39.56	69.77						
	Birth success (%)	0.9879	0.8774	1.098	0.9581	0.8475	1.069						
	Wean success, of all dams (%)	0.8830	0.6638	1.102	0.9061	0.6863	1.126						
	Wean success, of births (%)	0.8834	0.7079	1.059	0.9355	0.7567	1.114						
	Litter size	9.856	8.326	11.18	9.481	7.843	10.88						
	Sex ratio (# of females/total)	0.5207	0.4388	0.6026	0.5071	0.4218	0.5924						

Table 3.3. Continued.

	Variable	High Runner											
		Female						Male					
		No Wheel			Wheel			No Wheel			Wheel		
		LSM	LL	UL	LSM	LL	UL	LSM	LL	UL	LSM	LL	UL
Weaning	Body mass (g)	12.80	10.64	14.64	12.37	10.06	14.32	13.25	11.18	15.04	12.78	10.56	14.67
	Lean mass (g)	10.56	9.002	11.91	10.11	8.425	11.55	10.94	9.443	12.25	10.47	8.857	11.86
	Fat mass (g)	1.427	1.142	1.712	1.449	1.153	1.746	1.434	1.148	1.720	1.471	1.175	1.767
Wheel Testing	Body mass before wheels (g)	23.84	21.79	25.90	23.36	21.27	25.45	29.33	27.27	31.39	28.23	26.14	30.32
	Lean mass before wheels (g)	19.73	18.09	21.37	19.60	17.93	21.28	24.71	23.06	26.36	24.24	22.58	25.91
	Fat mass before wheels (g)	2.447	1.763	3.184	2.336	1.648	3.079	2.589	1.897	3.332	2.367	1.686	3.101
	Distance (revs/day)	13161	10887	15435	14085	11711	16459	12020	9702	14338	11739	9388	14090
	Duration (min)	553.1	460.6	645.6	562.6	468.6	656.5	518.1	424.9	611.3	505.8	412.2	599.4
	Avg. speed (revs/min)	23.55	19.73	27.37	25.14	21.17	29.10	23.08	19.20	26.95	23.02	19.08	26.96
	Max. speed (revs/min)	37.95	33.54	42.36	39.86	35.26	44.45	37.87	33.38	42.36	37.44	32.89	41.99
	Food consumption (g)	38.56	35.76	41.36	38.95	35.63	42.27	36.76	33.98	39.55	39.14	36.17	42.11
	Body mass after wheels (g)	23.69	21.53	25.86	23.47	21.28	25.66	28.75	26.58	30.92	27.88	25.69	30.07
	Lean mass after wheels (g)	19.78	17.97	21.59	19.62	17.77	21.46	24.80	22.99	26.62	24.24	22.40	26.07
	Fat mass after wheels (g)	2.040	1.399	2.736	1.924	1.283	2.625	1.683	1.078	2.348	1.613	1.014	2.275
Offspring Weaning	Body mass (g)	35.09	30.91	39.27	34.96	30.77	39.15						
	Pairing to offspring birth (days)	65.74	49.64	81.83	55.28	38.96	71.61						
	Birth success (%)	0.8333	0.6843	0.9823	0.9662	0.8111	1.121						
	Wean success, of all dams (%)	0.6345	0.3529	0.9161	0.8496	0.5618	1.137						
	Wean success, of births (%)	0.7880	0.5959	0.9801	0.8685	0.6759	1.061						
	Litter size	11.22	9.803	12.48	9.726	8.008	11.18						
	Sex ratio (# of females/total)	0.5096	0.4202	0.5990	0.5288	0.4334	0.6242						

Table 3.4. Birth and weaning success. Breeding pairs were established using the standard protocol for the HR selection experiment (see Section 2.1). For the offspring generation, breeders were also paired by maternal wheel access (i.e., females whose mothers had wheels were only paired with males whose mothers also had wheels). Cages were inspected daily from the time breeding pairs were established until offspring were weaned and any information pertinent for breeding success (e.g., if pairs needed to be separated due to fighting, litter loss prior to weaning) as noted. The total number of pairs, litters born, and litters surviving until weaning are presented as raw counts. Birth success (i.e., did the dam successfully give birth) was calculated as a 0-1 variable, and is presented as the average percent of litters born relative to the total number of pairs (including those that may have died prior to, or in the process of, giving birth) for each line. Wean success (i.e., did the dam successfully rear at least 1 pup until 21 days of age) was calculated as a 0-1 variable, and is presented in two ways: (1) as the average percent of litters surviving until weaning relative to the total number of pairs (including those that did not successfully get pregnant and/or give birth), and (2) the average percent of litters surviving until weaning relative to the numbers of successful births.

Variable	Maternal Generation															
	Control Lines								High Runner Lines							
	No Wheel				Wheel				No Wheel				Wheel			
	1	2	4	5	1	2	4	5	3	6	7	8	3	6	7	8
Total (N)	11	10	11	11	10	10	10	10	12	13	14	15	10	10	10	10
Deaths (N)	---	---	---	---	---	---	1	1	---	---	---	---	---	---	---	2
Births (N)	11	9	10	11	10	8	7	9	10	12	14	13	7	9	9	7
Weaned (N)	10	6	9	7	10	6	7	9	9	12	12	11	4	6	7	7
Births (%)	100.00	90.00	90.91	100.00	100.00	80.00	70.00	90.00	83.33	92.31	100.00	86.67	70.00	90.00	90.00	70.00
Weaned (% of total)	90.91	60.00	81.82	63.64	100.00	60.00	70.00	90.00	75.00	92.31	85.71	73.33	40.00	60.00	70.00	70.00
Weaned (% of births)	---	66.67	90.00	---	---	75.00	100.00	100.00	90.00	100.00	---	84.62	57.14	66.67	77.78	100.00
Variable	Offspring Generation															
	Control Lines								High Runner Lines							
	No Wheel				Wheel				No Wheel				Wheel			
	1	2	4	5	1	2	4	5	3	6	7	8	3	6	7	8
Total (N)	13	12	11	14	13	12	14	15	17	14	12	13	9	9	10	11
Deaths (N)	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Births (N)	12	10	11	13	12	12	14	15	14	12	10	9	6	9	10	9
Weaned (N)	12	9	10	11	11	12	13	13	12	11	10	9	6	8	10	7
Births (%)	92.31	83.33	100.00	92.86	92.31	100.00	100.00	100.00	82.35	85.71	83.33	69.23	66.67	100.00	100.00	81.82
Weaned (% of total)	92.31	75.00	90.91	78.57	84.62	100.00	92.86	86.67	70.59	78.57	83.33	69.23	66.67	88.89	100.00	63.64
Weaned (% of births)	100.00	90.00	---	84.62	91.67	---	---	---	85.71	91.67	100.00	100.00	100.00	---	---	77.78
Variable	Grand-Offspring Generation															
	Control Lines								High Runner Lines							
	No Wheel				Wheel				No Wheel				Wheel			
	1	2	4	5	1	2	4	5	3	6	7	8	3	6	7	8
Total (N)	9	6	8	8	6	9	7	7	11	8	8	7	4	7	6	7
Deaths (N)	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Births (N)	9	6	8	8	6	9	7	6	10	7	7	4	4	7	6	6
Weaned (N)	8	5	8	7	6	9	7	4	9	5	6	3	4	7	5	4
Births (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	85.71	90.91	87.50	87.50	57.14	100.00	100.00	100.00	85.71
Weaned (% of total)	88.89	83.33	100.00	87.50	100.00	100.00	100.00	57.14	81.82	62.50	75.00	42.86	100.00	100.00	83.33	57.14
Weaned (% of births)	---	---	---	---	---	---	---	66.67	90.00	71.43	85.71	75.00	---	---	---	66.67

Table 3.5. Least Squares Means (LSMs), F-statistics, and p-values from SAS Proc MIXED repeated-measures analyses with linetype and day as fixed effects, and age and wheel freeness as covariates. See Table 1 legend for further details.

Table 3.5. Continued.

Distance (revs/day)													
		Control			High Runner			Linetype		Day		Linetype*Day	
		LSM	LL	UL	LSM	LL	UL	F	P	F	P	F	P
Wheel Testing	Day 1	2419	1520	3317	6599	5210	7987	178.96	< 0.0001	39.24	< 0.0001	13.46	< 0.0001
	Day 2	3050	2141	3959	10035	8647	11423						
	Day 3	4064	3166	4962	12432	11055	13809						
	Day 4	4143	3244	5043	12899	11521	14277						
	Day 5	4960	4061	5860	14721	13343	16099						
	Day 6	5021	4121	5920	16779	15401	18157						
Running by Prospective Dams	Day 1	5201	4074	6329	11667	9344	13990	89.86	< 0.0001	10.16	< 0.0001	4.20	< 0.0001
	Day 2	4696	3569	5823	12993	10674	15312						
	Day 3	5301	4166	6435	15405	13092	17718						
	Day 4	5297	4170	6423	15296	12983	17609						
	Day 5	4694	3567	5821	12924	10611	15237						
	Day 6	6202	5075	7329	17945	15632	20258						
	Day 7	5633	4507	6760	17032	14719	19345						
	Day 8	6063	4938	7188	17646	15333	19959						
	Day 9	5826	4695	6957	16248	13935	18561						
	Day 10	5552	4431	6673	17383	15070	19696						
	Day 11	5759	4642	6877	16858	14545	19171						
	Day 12	6170	5059	7281	17304	14991	19617						
	Day 13	6197	5085	7308	16835	14522	19148						
	Day 14	6364	5252	7476	17449	15131	19767						
Running by Dams and Sires	Day 1	7211	6211	8211	16174	13939	18409	48.74	0.0004	3.31	< 0.0001	1.65	0.0659
	Day 2	6219	5232	7207	16297	14055	18539						
	Day 3	6584	5601	7567	16007	13770	18244						
	Day 4	6299	5333	7265	13832	11604	16060						
	Day 5	6736	5770	7703	14325	12096	16554						
	Day 6	6478	5512	7445	12904	10675	15133						
	Day 7	5990	5025	6955	12777	10541	15013						
	Day 8	5773	4811	6736	12812	10583	15041						
	Day 9	5849	4893	6805	11783	9553	14013						
	Day 10	6035	5092	6978	11908	9679	14137						
	Day 11	5917	4959	6874	12357	10129	14585						
	Day 12	5901	4937	6865	11541	9316	13766						
	Day 13	5208	4242	6173	12148	9913	14383						
	Day 14	5984	5021	6946	12466	10230	14702						
	Day 15	5367	4413	6322	11950	9683	14217						
	Day 16	5425	4469	6382	11892	9648	14136						
	Day 17	5663	4710	6616	12294	10066	14522						
	Day 18	5895	4934	6856	12175	9931	14419						
Running Before Birth (Dams Only)	Day 1	4108	2135	6081	8766	6834	10698	13.42	0.0105	8.08	< 0.0001	2.01	0.0334
	Day 2	5371	3826	6917	6992	4847	9137						
	Day 3	5277	3740	6815	8443	6515	10371						
	Day 4	3498	1840	5156	8235	6306	10164						
	Day 5	2574	920	4229	7489	5572	9406						
	Day 6	3451	1920	4983	7359	5477	9241						
	Day 7	4431	2900	5962	6149	4358	7940						
	Day 8	4051	2537	5564	4634	3109	6159						
	Day 9	2762	1299	4224	4805	3315	6295						
	Day 10	3071	1757	4386	4898	3500	6296						
	Day 11	2512	1314	3710	3358	2139	4577						
	Day 12	2277	1480	3073	2580	1506	3653						
	Day 13	1541	834	2248	1935	914	2957						
	Day 14	1219	625	1813	1926	939	2912						
	Day 15	965	356	1575	2199	1136	3262						
	Day 16	No dams running days 16-18											
	Day 17												
	Day 18												
Running After Birth (with pups)	Day 1	1472	1012	2073	2170	1405	3209	0.63	0.4585	3.05	0.0051	0.75	0.6658
	Day 2	1118	743	1619	1040	629	1626						
	Day 3	1227	839	1738	1461	938	2178						
	Day 4	1337	921	1881	1607	1039	2382						
	Day 5	1460	1012	2042	1410	900	2111						
	Day 6	1195	813	1696	1192	747	1811						
	Day 7	1102	744	1576	1252	788	1897						
	Day 8	1082	729	1549	1312	831	1976						
	Day 9	956	634	1386	1353	862	2030						
	Day 10	829	542	1218	1157	718	1773						

Table 3.5. Continued.

Duration (min)													
		Control			High Runner			Linetype		Day		Linetype*Day	
		LSM	LL	UL	LSM	LL	UL	F	P	F	P	F	P
Wheel Testing	Day 1	382.3	328.6	436.0	513.8	448.2	579.4	19.60	0.0044	8.80	< 0.0001	0.46	0.8019
	Day 2	397.0	342.9	451.2	548.4	482.9	614.0						
	Day 3	466.5	412.8	520.2	610.3	545.4	675.3						
	Day 4	438.9	385.2	492.6	601.7	536.7	666.7						
	Day 5	494.4	440.7	548.1	622.1	557.1	687.0						
	Day 6	494.5	440.8	548.2	652.5	587.5	717.5						
Running by Prospective Dams	Day 1	523.2	475.0	571.5	583.3	523.1	643.5	7.18	0.0365	6.35	< 0.0001	0.88	0.5761
	Day 2	441.6	393.4	489.9	541.6	481.5	601.7						
	Day 3	478.7	430.2	527.1	591.8	532.1	651.6						
	Day 4	460.6	412.4	508.9	554.6	494.8	614.3						
	Day 5	410.1	361.8	458.4	528.1	468.4	587.9						
	Day 6	530.1	481.9	578.4	592.8	533.0	652.5						
	Day 7	471.4	423.2	519.7	565.3	505.5	625.0						
	Day 8	480.4	432.2	528.7	557.3	497.6	617.0						
	Day 9	464.0	415.6	512.5	534.6	474.9	594.3						
	Day 10	454.2	406.1	502.4	549.2	489.5	608.9						
	Day 11	464.4	416.3	512.5	541.8	482.0	601.5						
	Day 12	485.3	437.4	533.1	531.0	471.2	590.7						
	Day 13	467.9	420.1	515.8	525.3	465.5	585.0						
	Day 14	472.1	424.2	520.0	548.4	488.3	608.4						
Running by Dams and Sires	Day 1	741.3	675.4	807.1	765.9	673.3	858.5	0.68	0.4401	6.09	< 0.0001	1.61	0.0759
	Day 2	641.6	575.9	707.2	683.6	590.7	776.5						
	Day 3	666.3	600.8	731.8	704.4	611.7	797.0						
	Day 4	633.0	568.0	698.1	657.4	565.1	749.7						
	Day 5	678.5	613.4	743.5	676.1	583.7	768.4						
	Day 6	664.9	599.9	730.0	644.3	551.9	736.6						
	Day 7	627.0	561.9	692.0	661.0	568.4	753.7						
	Day 8	605.8	540.8	670.8	658.9	566.6	751.3						
	Day 9	614.3	549.3	679.2	643.8	551.5	736.2						
	Day 10	631.6	566.9	696.2	638.8	546.4	731.2						
	Day 11	626.0	561.1	691.0	675.9	583.6	768.3						
	Day 12	623.7	558.6	688.7	651.6	559.3	743.8						
	Day 13	553.9	488.9	618.9	683.3	590.7	775.9						
	Day 14	620.0	554.9	685.0	671.0	578.4	763.7						
	Day 15	555.7	490.8	620.6	632.2	538.4	725.9						
	Day 16	570.5	505.5	635.5	641.2	548.3	734.2						
	Day 17	585.4	520.5	650.3	641.7	549.2	734.1						
	Day 18	580.5	515.5	645.5	644.2	551.2	737.2						
Running Before Birth (Dams Only)	Day 1	335.4	198.0	472.7	525.3	384.1	666.5	1.95	0.2118	7.13	< 0.0001	1.40	0.1830
	Day 2	437.0	325.8	548.1	471.4	318.0	624.7						
	Day 3	426.7	316.1	537.2	518.6	378.2	659.1						
	Day 4	337.6	220.2	454.9	502.7	362.7	642.6						
	Day 5	328.0	210.9	445.0	472.2	333.8	610.5						
	Day 6	360.7	251.1	470.3	474.4	339.5	609.2						
	Day 7	368.1	259.2	477.0	436.2	308.7	563.7						
	Day 8	411.4	304.6	518.2	353.9	242.8	464.9						
	Day 9	310.2	208.1	412.3	397.5	289.8	505.1						
	Day 10	352.1	260.8	443.5	420.3	319.9	520.7						
	Day 11	322.1	240.4	403.9	288.6	199.3	377.8						
	Day 12	259.0	202.9	315.0	275.1	194.3	355.8						
	Day 13	192.7	142.6	242.7	228.2	150.7	305.7						
	Day 14	183.9	140.6	227.2	218.3	142.8	293.9						
	Day 15	124.0	79.8	168.3	155.7	75.7	235.8						
	Day 16	No dams running days 16-18											
	Day 17												
	Day 18												
Running After Birth (with pups)	Day 1	160.7	124.4	201.5	154.5	111.8	204.2	0.03	0.8633	2.61	0.0140	0.71	0.6999
	Day 2	159.2	122.7	200.5	126.2	89.7	168.9						
	Day 3	172.1	135.6	212.9	153.5	114.3	198.4						
	Day 4	170.6	134.3	211.3	168.0	126.5	215.3						
	Day 5	178.6	141.1	220.4	154.4	115.0	199.7						
	Day 6	146.4	112.8	184.3	130.5	94.6	172.0						
	Day 7	130.7	99.1	166.6	129.9	94.0	171.6						
	Day 8	122.6	92.1	157.5	129.3	93.6	170.8						
	Day 9	103.8	75.8	136.3	129.2	93.6	170.5						
	Day 10	96.2	69.3	127.6	119.9	85.2	160.5						

Table 3.5. Continued.

Avg. Speed (revs/min)													
		Control			High Runner			Linetype		Day		Linetype*Day	
		LSM	LL	UL	LSM	LL	UL	F	P	F	P	F	P
Wheel Testing	Day 1	6.32	4.64	8.00	12.37	9.97	14.77	78.31	< 0.0001	54.95	< 0.0001	16.46	< 0.0001
	Day 2	7.60	5.92	9.28	18.11	15.71	20.51						
	Day 3	8.75	7.07	10.42	20.29	17.90	22.69						
	Day 4	9.60	7.92	11.28	21.52	19.13	23.92						
	Day 5	10.31	8.63	11.99	23.86	21.47	26.26						
	Day 6	10.36	8.68	12.04	26.00	23.60	28.39						
Running by Prospective Dams	Day 1	9.79	8.26	11.32	20.26	15.09	25.42	42.96	0.0006	19.68	< 0.0001	10.12	< 0.0001
	Day 2	10.35	8.82	11.88	24.27	19.11	29.43						
	Day 3	10.82	9.28	12.35	26.20	21.05	31.36						
	Day 4	11.19	9.66	12.72	27.64	22.48	32.79						
	Day 5	11.00	9.47	12.53	24.26	19.10	29.41						
	Day 6	11.61	10.08	13.14	30.05	24.90	35.21						
	Day 7	11.73	10.20	13.25	29.98	24.82	35.14						
	Day 8	12.35	10.82	13.87	31.55	26.40	36.71						
	Day 9	12.26	10.73	13.79	30.26	25.10	35.42						
	Day 10	12.03	10.50	13.55	31.33	26.17	36.48						
	Day 11	12.19	10.67	13.71	31.17	26.01	36.33						
	Day 12	12.64	11.13	14.16	32.49	27.34	37.65						
	Day 13	13.11	11.59	14.62	32.23	27.07	37.39						
	Day 14	13.12	11.61	14.63	32.44	27.28	37.60						
Running by Dams and Sires	Day 1	9.67	8.68	10.68	20.74	17.80	23.78	52.57	0.0003	2.87	0.0005	2.59	0.0017
	Day 2	9.73	8.75	10.73	23.51	20.47	26.66						
	Day 3	9.89	8.91	10.89	22.32	19.32	25.42						
	Day 4	10.04	9.07	11.03	20.47	17.55	23.50						
	Day 5	9.97	9.00	10.96	20.53	17.61	23.57						
	Day 6	9.84	8.87	10.82	19.67	16.78	22.67						
	Day 7	9.62	8.67	10.60	19.01	16.14	21.99						
	Day 8	9.59	8.64	10.57	19.25	16.37	22.23						
	Day 9	9.63	8.68	10.59	17.97	15.15	20.91						
	Day 10	9.61	8.68	10.57	18.25	15.42	21.20						
	Day 11	9.53	8.58	10.50	17.99	15.17	20.93						
	Day 12	9.53	8.58	10.51	17.53	14.73	20.45						
	Day 13	9.43	8.48	10.41	17.54	14.74	20.47						
	Day 14	9.75	8.79	10.73	18.34	15.50	21.30						
	Day 15	9.66	8.72	10.64	18.74	15.86	21.73						
	Day 16	9.51	8.57	10.47	18.40	15.55	21.37						
	Day 17	9.73	8.79	10.70	19.09	16.22	22.07						
	Day 18	10.13	9.17	11.12	18.85	15.98	21.83						
Running Before Birth (Dams Only)	Day 1	11.72	7.80	15.64	16.62	11.38	21.85	8.87	0.0247	2.13	0.0238	0.77	0.6962
	Day 2	12.21	9.09	15.33	14.78	9.33	20.23						
	Day 3	12.08	8.98	15.19	16.45	11.23	21.67						
	Day 4	10.46	7.17	13.75	15.87	10.67	21.08						
	Day 5	7.95	4.64	11.26	16.07	10.89	21.25						
	Day 6	9.21	6.13	12.29	15.34	10.21	20.46						
	Day 7	11.03	7.96	14.09	13.44	8.45	18.44						
	Day 8	9.58	6.57	12.58	12.22	8.12	16.31						
	Day 9	8.87	5.99	11.75	11.68	7.65	15.72						
	Day 10	8.44	5.87	11.01	10.70	6.78	14.61						
	Day 11	8.05	5.74	10.36	10.82	7.50	14.14						
	Day 12	8.40	6.79	10.01	9.37	6.24	12.50						
	Day 13	7.58	6.13	9.04	8.73	5.66	11.81						
	Day 14	7.10	5.83	8.37	7.24	4.17	10.32						
	Day 15	7.88	6.58	9.17	12.64	9.52	15.77						
	Day 16	No dams running days 16-18											
	Day 17												
	Day 18												
Running After Birth (with pups)	Day 1	9.80	7.53	12.21	15.24	12.81	17.77	2.50	0.1647	5.70	< 0.0001	1.42	0.2014
	Day 2	7.58	5.45	9.87	9.02	6.99	11.17						
	Day 3	7.67	5.58	9.92	10.72	8.65	12.89						
	Day 4	8.51	6.36	10.82	10.68	8.61	12.87						
	Day 5	9.04	6.84	11.38	10.29	8.24	12.44						
	Day 6	9.00	6.81	11.33	10.07	8.05	12.21						
	Day 7	9.17	6.96	11.51	10.49	8.43	12.66						
	Day 8	9.41	7.19	11.77	10.91	8.83	13.08						
	Day 9	9.91	7.66	12.31	11.38	9.29	13.57						
	Day 10	9.36	7.14	11.72	10.65	8.57	12.84						

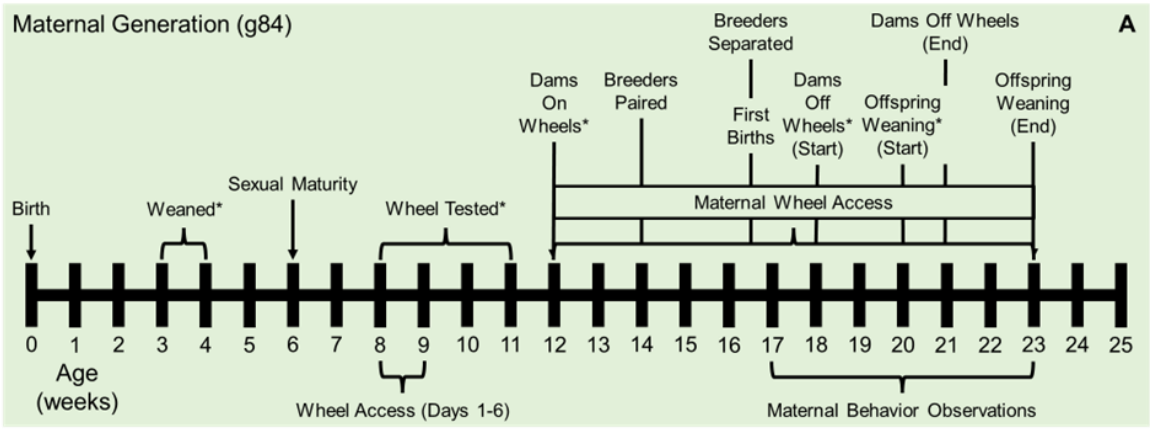
Table 3.6. Frequencies of observed behaviors. Behavioral observations were conducted three times daily, corresponding to known periods of activity behavior in the HR mice (i.e., high activity 1-hour prior to lights on, low activity mid-day, high activity 1-hour after lights off), from offspring birth until weaning. Behaviors are organized by their assigned categories: “maternal care,” “activity,” and “maintenance.” Behaviors are also partitioned into several observational periods, corresponding to observations made in the days after birth, and the morning, afternoon, and evening observations. Behaviors are presented as the average fraction of observations relative to the total number of observations in that period (e.g., post-partum days 1-5 has 15 observations, 3x daily across 5 days, so a value of 0.40 for a given behavior would equate to 6 of 15 observations).

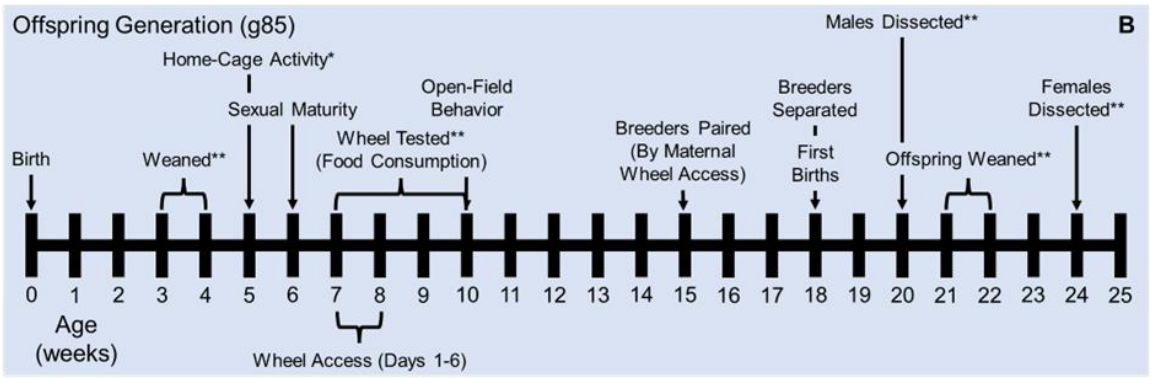
Behavior	Post-Partum Days 1-5				Post-Partum Days 6-10				Post-Partum Days 11-15				Post-Partum Days 16-20			
	Control		High Runner		Control		High Runner		Control		High Runner		Control		High Runner	
	No Wheel	Wheel	No Wheel	Wheel	No Wheel	Wheel	No Wheel	Wheel	No Wheel	Wheel	No Wheel	Wheel	No Wheel	Wheel	No Wheel	Wheel
Resting with Pups	0.0261	0.0162	0.0347	0.0142	0.0071	0.0184	0.0076	0.0074	0.0208	0.0334	0.0168	0.0108	0.1516	0.1263	0.0848	0.0667
Grooming Pups	0.0164	0.0088	0.0173	0.0071	0.0048	0.0053	0.0038	0.0074	0.0033	0.0109	---	---	0.0017	0.0018	0.0090	---
Carrying Pups	---	0.0018	---	0.0053	---	---	---	0.0070	---	---	---	---	---	---	---	---
Nursing	0.4337	0.4058	0.4913	0.3228	0.3254	0.3916	0.4113	0.2708	0.3598	0.3907	0.3713	0.2896	0.2032	0.2193	0.2584	0.2054
Maternal Care	0.4762	0.4325	0.5433	0.3494	0.3372	0.4153	0.4227	0.2926	0.3839	0.4350	0.3881	0.3004	0.3564	0.3474	0.3522	0.2721
Digging	0.0280	0.0070	0.0076	0.0053	0.0050	0.0128	0.0038	0.0018	0.0017	0.0132	0.0013	0.0036	0.0017	0.0053	---	0.0036
Grooming Self	0.0636	0.0477	0.0226	0.0441	0.0603	0.0487	0.0438	0.0488	0.0570	0.0534	0.0566	0.0509	0.0616	0.0895	0.0564	0.0378
Walking	0.1232	0.0407	0.1532	0.1204	0.1285	0.0267	0.1614	0.1398	0.1343	0.0725	0.1390	0.1838	0.1317	0.0719	0.1940	0.2144
Climbing	0.0098	---	0.0177	0.0018	---	0.0035	0.0198	0.0175	0.0073	0.0132	0.0065	0.0479	0.0133	0.0105	0.0392	0.0505
Sniffing	0.0114	0.0123	0.0224	0.0140	0.0253	0.0182	0.0340	0.0260	0.0275	0.0236	0.0362	0.0244	0.0217	0.0140	0.0205	0.0072
Standing	0.0767	0.0212	0.0713	0.0194	0.0892	0.0380	0.0986	0.0279	0.0845	0.1013	0.1170	0.1373	0.1418	0.1351	0.1210	0.1171
Chewing	0.0260	---	0.0280	---	0.0315	0.0105	0.0149	0.0073	0.0178	0.0530	0.0164	0.0380	0.0400	0.0912	0.0487	0.0865
Non-Running Activity	0.3386	0.1289	0.3228	0.2050	0.3398	0.1583	0.3762	0.2691	0.3301	0.3300	0.3731	0.4860	0.4117	0.4175	0.4798	0.5171
Running	---	0.2974	---	0.2099	0.0015	0.2189	---	0.2045	---	---	---	---	---	---	---	---
All Activity	0.3386	0.4263	0.3228	0.4149	0.3413	0.3773	0.3762	0.4735	0.3301	0.3300	0.3731	0.4860	0.4117	0.4175	0.4798	0.5171
Resting Alone	0.0877	0.0511	0.0662	0.1462	0.1295	0.0571	0.0923	0.1390	0.1118	0.1046	0.1142	0.1182	0.1251	0.1281	0.0937	0.1568
Resting in Wheel	---	0.0195	---	0.0070	---	0.0249	---	0.0147	---	---	---	---	---	---	---	---
Eating	0.0894	0.0653	0.0601	0.0649	0.1681	0.1020	0.0926	0.0641	0.1499	0.1174	0.1068	0.0864	0.0916	0.0930	0.0667	0.0432
Drinking	0.0114	0.0053	0.0062	0.0158	0.0220	0.0181	0.0162	0.0143	0.0242	0.0129	0.0153	0.0090	0.0151	0.0140	0.0064	0.0090
Inactive/Maintenance	0.1884	0.1412	0.1325	0.2340	0.3196	0.2022	0.2011	0.2321	0.2860	0.2350	0.2362	0.2136	0.2318	0.2351	0.1668	0.2090

Behavior	All				Morning				Afternoon				Evening			
	Control		High Runner		Control		High Runner		Control		High Runner		Control		High Runner	
	No Wheel	Wheel	No Wheel	Wheel	No Wheel	Wheel	No Wheel	Wheel	No Wheel	Wheel	No Wheel	Wheel	No Wheel	Wheel	No Wheel	Wheel
Resting with Pups	0.0513	0.0487	0.0355	0.0246	0.0559	0.0527	0.0278	0.0211	0.0847	0.0789	0.0612	0.0457	0.0146	0.0159	0.0185	0.0079
Grooming Pups	0.0066	0.0067	0.0075	0.0036	0.0025	0.0053	0.0065	0.0053	0.0064	0.0069	0.0097	0.0055	0.0109	0.0080	0.0065	---
Carrying Pups	---	0.0005	---	0.0031	---	0.0013	---	0.0053	---	---	---	0.0014	---	---	---	0.0026
Nursing	0.3321	0.3516	0.3810	0.2713	0.3080	0.3368	0.3556	0.2429	0.5250	0.5527	0.6001	0.4594	0.1724	0.1742	0.1976	0.1200
Maternal Care	0.3900	0.4075	0.4240	0.3025	0.3665	0.3962	0.3898	0.2745	0.6161	0.6384	0.6710	0.5120	0.1980	0.1980	0.2227	0.1305
Digging	0.0105	0.0094	0.0031	0.0036	0.0074	0.0092	0.0046	0.0026	0.0040	0.0141	0.0010	0.0083	0.0201	0.0053	0.0038	---
Grooming Self	0.0614	0.0598	0.0446	0.0453	0.0706	0.0580	0.0421	0.0422	0.0616	0.0693	0.0566	0.0547	0.0515	0.0528	0.0358	0.0395
Walking	0.1289	0.0528	0.1619	0.1630	0.1456	0.0514	0.1918	0.1980	0.0526	0.0319	0.0340	0.0352	0.1846	0.0742	0.2537	0.2495
Climbing	0.0074	0.0067	0.0214	0.0293	0.0073	0.0079	0.0165	0.0211	0.0013	---	0.0010	0.0014	0.0134	0.0118	0.0459	0.0642
Sniffing	0.0211	0.0170	0.0281	0.0176	0.0268	0.0197	0.0398	0.0296	0.0051	0.0069	0.0068	0.0070	0.0307	0.0237	0.0367	0.0158
Standing	0.0971	0.0738	0.1025	0.0746	0.0942	0.0777	0.1086	0.0945	0.0128	0.0125	0.0116	0.0082	0.1801	0.1287	0.1820	0.1181
Chewing	0.0285	0.0387	0.0281	0.0324	0.0316	0.0237	0.0276	0.0256	---	0.0027	---	0.0028	0.0523	0.0882	0.0548	0.0676
Non-Running Activity	0.3550	0.2582	0.3898	0.3658	0.3835	0.2476	0.4311	0.4137	0.1375	0.1375	0.1110	0.1176	0.5328	0.3846	0.6125	0.5546
Running	---	0.1297	---	0.1077	---	0.1596	---	0.1348	---	0.0097	---	0.0055	0.0012	0.2143	---	0.1775
All Activity	0.3554	0.3879	0.3898	0.4735	0.3835	0.4071	0.4311	0.5485	0.1375	0.1472	0.1110	0.1232	0.5339	0.5990	0.6125	0.7321
Resting Alone	0.1134	0.0853	0.0933	0.1414	0.1045	0.0740	0.0689	0.0835	0.1744	0.1523	0.1801	0.3169	0.0651	0.0329	0.0367	0.0330
Resting in Wheel	---	0.0111	---	0.0053	---	0.0079	---	0.0053	---	0.0042	---	0.0014	---	0.0211	---	0.0092
Eating	0.1234	0.0943	0.0807	0.0639	0.1188	0.1003	0.0917	0.0738	0.0616	0.0455	0.0311	0.0373	0.1872	0.1345	0.1170	0.0794
Drinking	0.0178	0.0126	0.0110	0.0121	0.0292	0.0118	0.0157	0.0132	0.0077	0.0125	0.0058	0.0068	0.0158	0.0132	0.0111	0.0158
Inactive/Maintenance	0.2546	0.2033	0.1850	0.2227	0.2524	0.1940	0.1763	0.1756	0.2437	0.2144	0.2169	0.3623	0.2681	0.2017	0.1648	0.1374

Figure 3.1. Experimental timelines for the (A) maternal, (B) offspring, and (C) grand-offspring generations. Each generation followed standard protocol for the HR mouse selection protocol: (1) mice are weaned at 3 weeks of age, (2) given access to a running wheel at sexual maturity (starting at 6-8 weeks of age) for a 6-day period to select breeders, (3) breeders are paired within line (i.e., C line 1 females are paired with C lines 1 males), (4) males are removed after 18 days, (5) offspring are weaned at 3 weeks of age. Times when body mass was recorded are noted with an asterisk and times when body composition (lean and fat mass) was also recorded are noted with two asterisks. Alterations to the standard HR selection protocol are two-fold: (1) 80 prospective dams (40 HR and 40 C) were given access to wheels from 2 weeks prior to breeding through 10 days after offspring birth, and (2) within the offspring generation, breeding pairs were made within line (as normal) and by maternal wheel access (i.e., females whose mothers had wheels were only paired with males whose mothers also had wheels).

Figure 3.1. Continued.





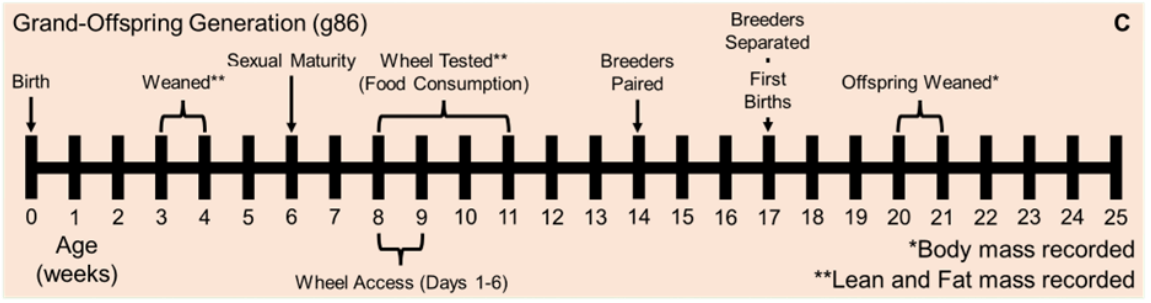


Figure 3.2. Maternal wheel-running (A) distance, (B) duration, (C) average speed, and (D) maximum speed. Data are partitioned into 5 broad periods or phases, occurring during: (1) across all 6 days of the standard 6-day test to select breeders, (2) the 2-week period when females were individually housed, (3) the 18-day period when males were present (wheel running during this period may represent running by the male, female or both), (4) the 18-day period before birth (wheel running during this period is synchronized to offspring birth, as some dams may have gotten pregnant within the first 24-hours after males were added, while others may have gotten pregnant in the final 24-hours before males were removed), (5) the 10-day period after birth (i.e., with pups). Data correspond to the daily average value for each line (C lines depicted in blue hues, HR lines depicted in orange/red hues). P-values are from SAS Proc MIXED repeated-measures analyses, with an autoregressive covariance structure, linetype and day as fixed effects, and age and wheel freeness as covariates. Results from these analyses can be found in Table 3.6.

Figure 3.2. Continued.

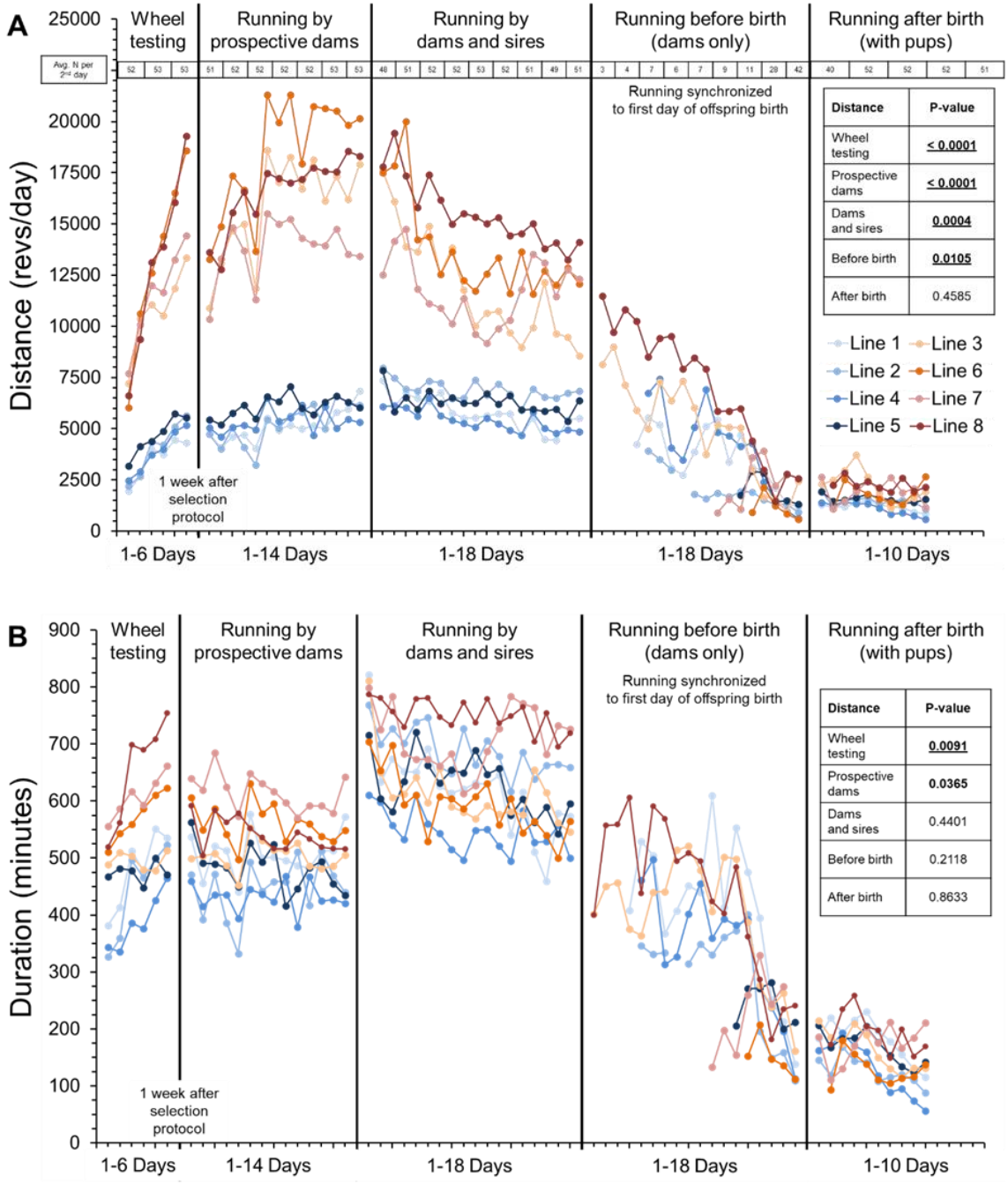


Figure 3.2. Continued.

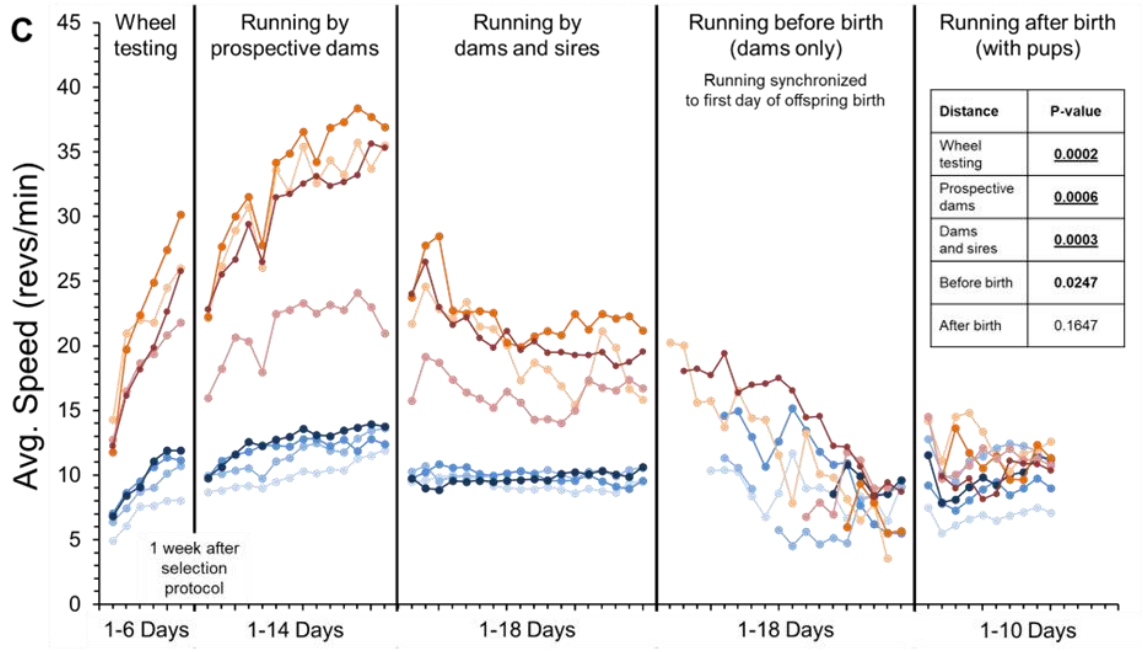


Figure 3.3. Wheel-running during the standard 6-day testing period: (A) distance, (B) duration, (C) average speed, and (D) maximum speed. Wheel revolutions are recorded in 1-minute intervals over a period of 23 hours every day, after which we calculated the number of revolutions (i.e., daily running distance), number of 1-minute intervals with at least one revolution (i.e., minutes of wheel activity; duration), the mean revolutions per minute (i.e., average running speed), and maximum revolutions in any minute (i.e., maximum running speed). Data are presented as LSMs and associated 95% CL. For the maternal generation, these data were measured prior to maternal wheel access, and thus were analyzed using SAS Proc MIXED with linetype and sex as fixed effects. For the offspring and grand-offspring generations, data were analyzed with linetype, sex, and maternal wheel access as fixed effects (C lines are blue, whereas HR lines are red; no wheel dams have slashed bars, whereas wheel dams have solid bars; males are in darker hues, females in lighter hues). In each generation, wheel-running traits were analyzed with age and a measure of wheel freeness as covariates. Results from these analyses can be found in Tables 3.1-3.3: Wheel Testing.

Figure 3.3. Continued.

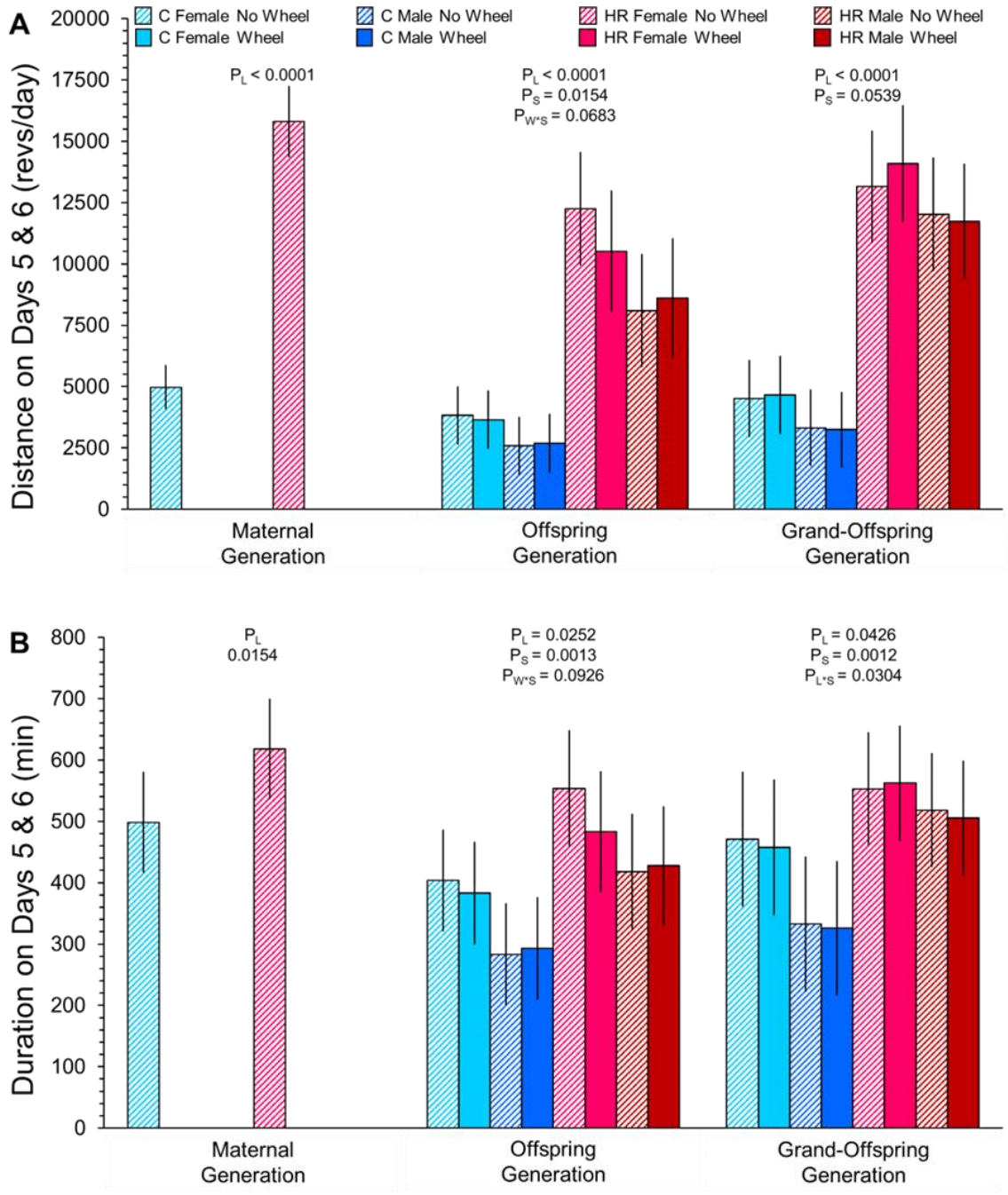


Figure 3.3. Continued.

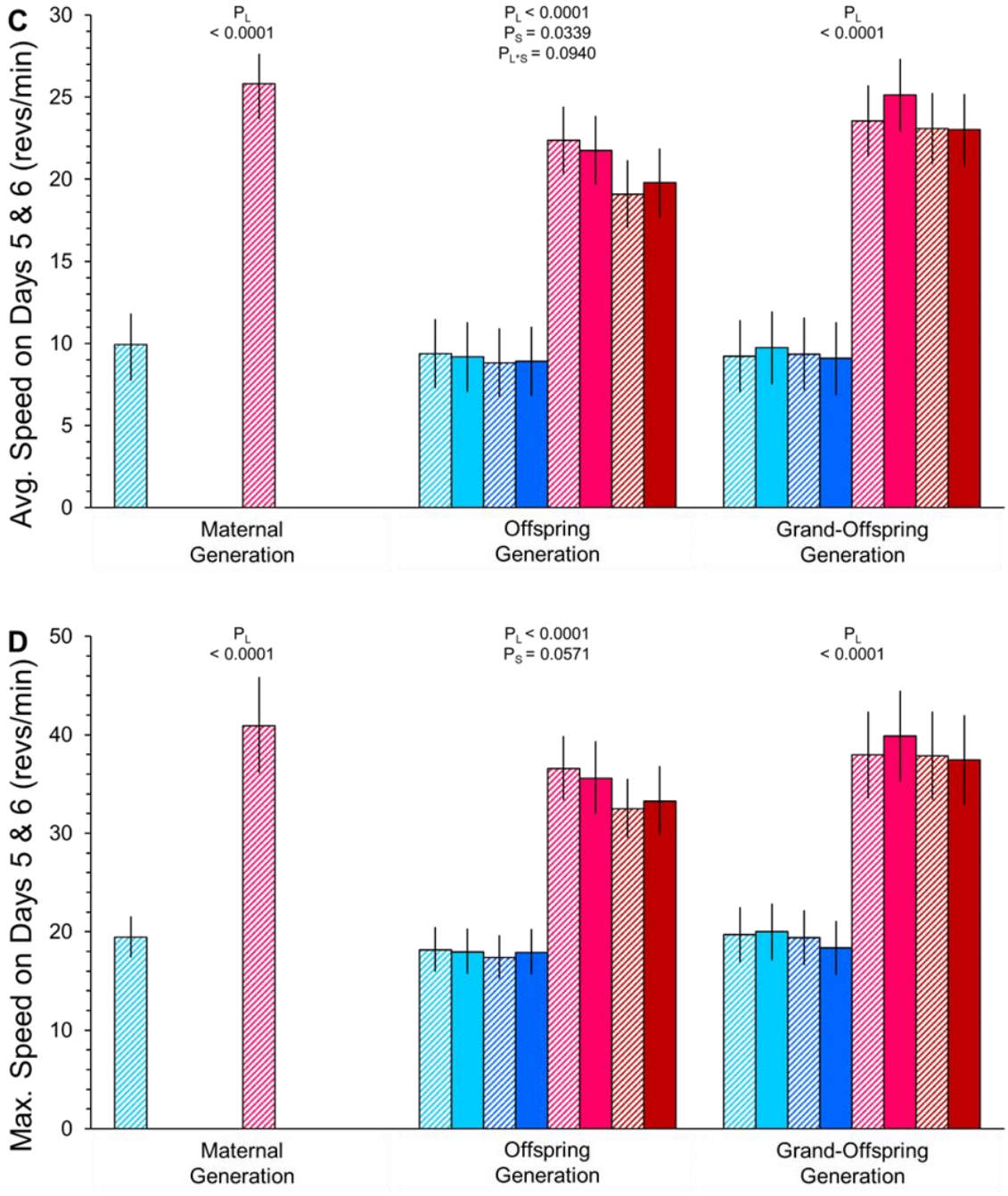


Figure 3.4. Body mass measured across generations; (A) maternal), (B) offspring, and (C) grand-offspring generations. Body mass is routinely measured as part of the standard selection protocol at: weaning (21 days of age), at the start of wheel access (~6-8 eeks of age, i.e., sexual maturity), at the end of wheel access (1 week later), and for female mice, at their offspring's weaning. Additionally, "Moms On Wheels" and "Moms Off Wheels" correspond to body mass measured before and after the maternal wheel access period (see Figure 2). "Moms On Wheels" occurs after the standard 6-day wheel testing period, and before the running by prospective dams. "Moms Off Wheels" occurs after the maternal wheel access period (i.e., "running after birth" in Figure 2), but is 10 days prior to offspring weaning. Data are presented as LSMs and associated 95% CL. For (A), body mass prior to maternal wheel access (i.e., at weaning, at standard 6-day testing period, and before being placed on wheels again) was analyzed using SAS Proc MIXED with linetype and sex as fixed effects (results for males are not shown, but can be found in Table 1). After maternal wheel access, body mass was analyzed with linetype and wheel access as fixed effects. For (B) and (C), all measures of body mass were analyzed using SAS Proc MIXED with linetype, sex, and maternal wheel access as fixed effects, except for body mass at offspring weaning (consisting of only females), which were analyzed with linetype and maternal wheel access as fixed effects (C lines are blue, whereas HR lines are in red; no wheel dams have slashed bars, whereas wheel dams have solid bars; males are in darker hues, females in lighter hues). Results from these analyses can be found throughout Tables 3.1-3.3. P-values (with corresponding main effect or interaction as subscript: L = Linetype, S = Sex, W = Wheel access) are listed within the figure.

Figure 3.4. Continued.

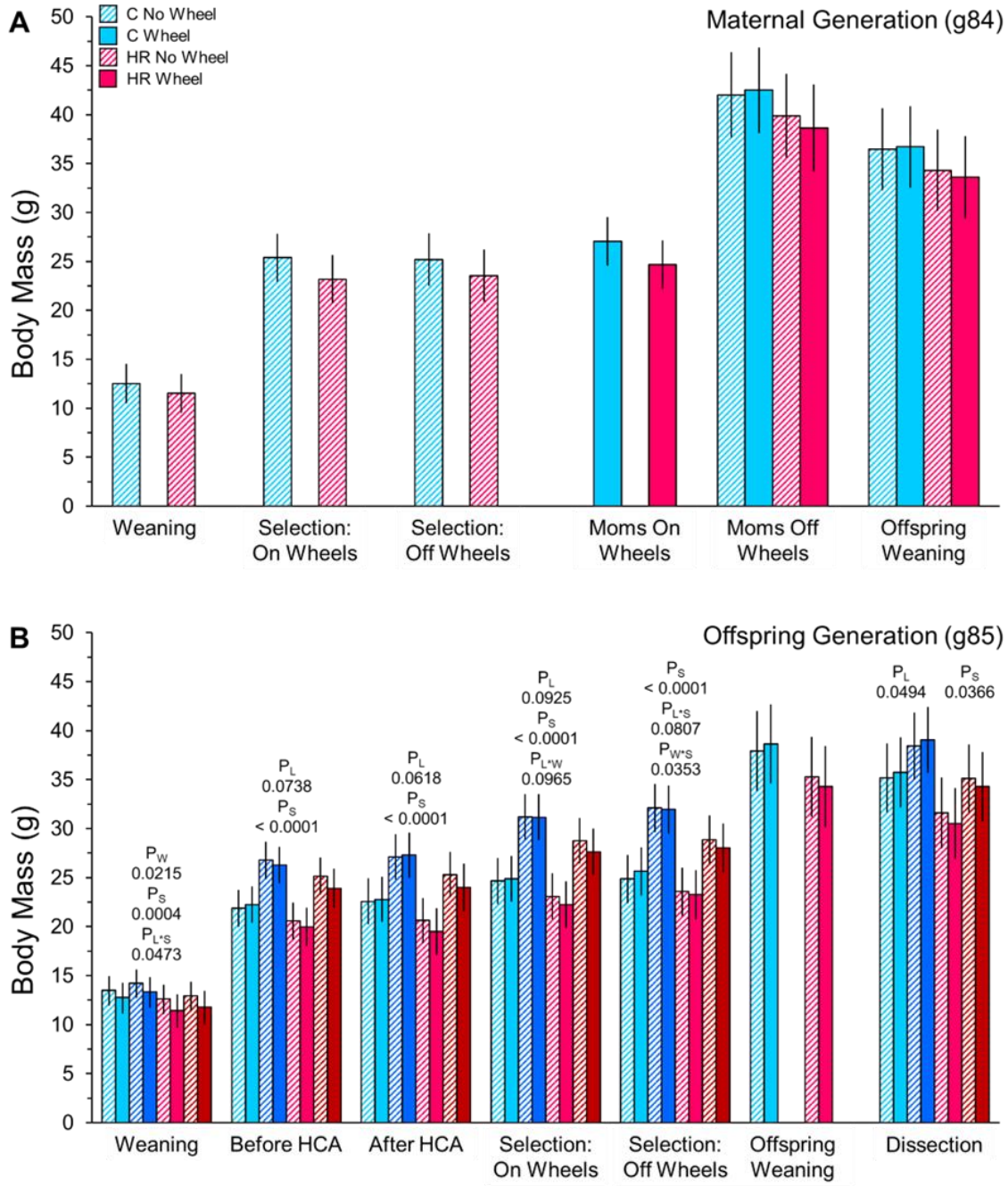
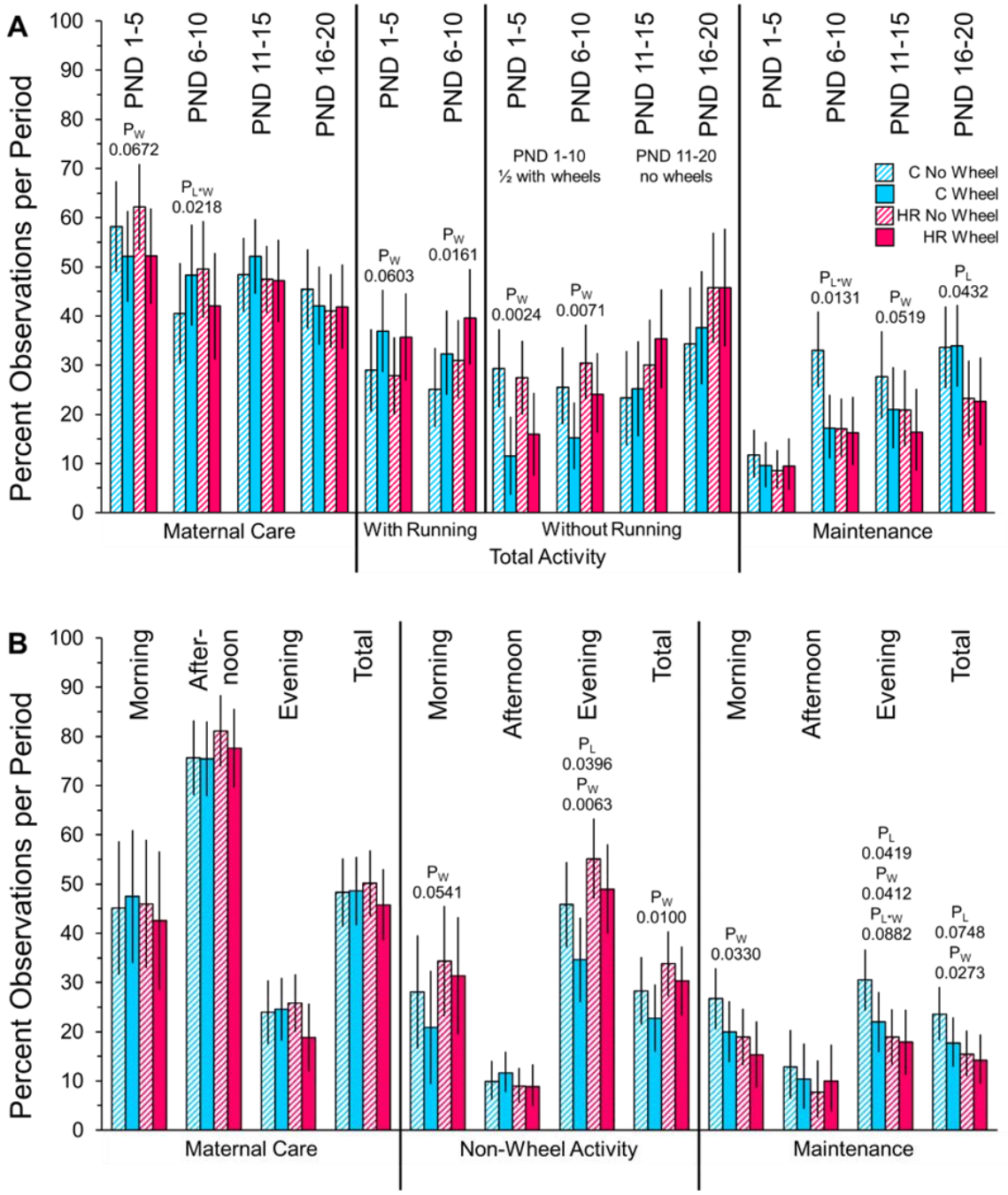


Figure 3.4. Continued.

Figure 3.5. Maternal behavior observations during (A) Post-Natal Days 1-20, (B) the morning, afternoon, and evening, and (C) across all observations. Maternal behavior was recorded three times daily, corresponding to known periods of activity behavior (i.e., high activity 1-hour prior to lights on, low activity mid-day, high activity 1-hour after lights off), from offspring birth until weaning. Behaviors were assigned to one of three pre-determined categories: “maternal care”, “activity”, and “maintenance”. Behavior during (A) PND 1-20 and (B) the morning, afternoon, and evening, are presented as a percent of observations for each period (i.e., PND 1-5 corresponds to 3x daily observations across 5 days, “morning” corresponds to 1x daily observations across 20 days), while (C) shows the frequency (as a percentage of total observations) of each observed behavior. Data are presented as Least Squares Means (LSMs) with associated 95% confidence limits from SAS Proc MIXED analyses, with linetype and wheel access as fixed effects, and age and litter size (which was a significant positive predictor of nursing behavior) as covariates (C lines are blue, whereas HR lines are red; no wheel dams have slashed bars while wheel dams have solid bars). Results from these analyses can be found in Table 1: Maternal Behavior.

Figure 3.5. Continued.



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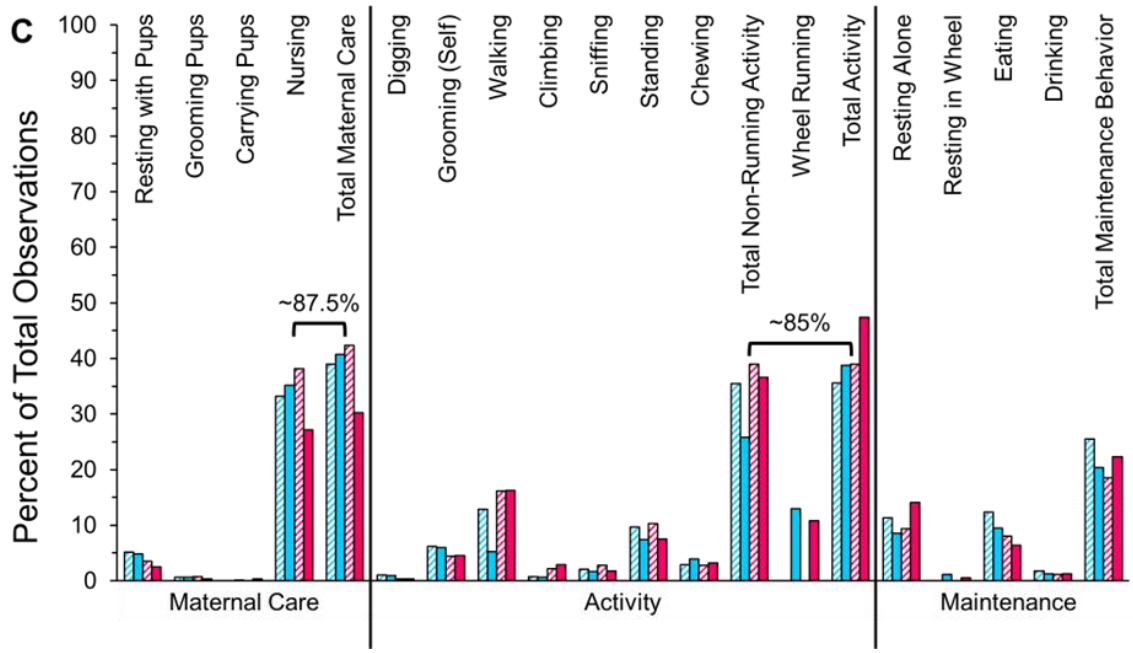


Figure 3.6. Reproductive success for the (A) maternal, (B) offspring, and (C) grand-offspring generations. Breeding pairs were established using the standard protocol for the HR selection experiment (see Section 2.1). For the offspring generation, breeders were also paired by maternal wheel access (i.e., females whose mothers had wheels were only paired with males whose mothers also had wheels). Cages were inspected daily from the time breeding pairs were established until offspring were weaned and any information pertinent for breeding success (e.g., if pairs needed to be separated due to fighting, litter loss to weaning) as noted. Data are presented as averages calculated by line (where the 4 bars per group correspond to C lines 1, 2, 4, and 5 or HR lines 3, 6, 7, and 8, displayed in that order). Data were analyzed using SAS GLIMMIX, with linetype and maternal wheel access as fixed effects (C lines are blue, whereas HR lines are red; no wheel dams have slashed bars and wheel dams have solid bars). Results from these analyses can be found in Tables 3.1-3.3: Offspring Weaning.

Figure 3.6. Continued.

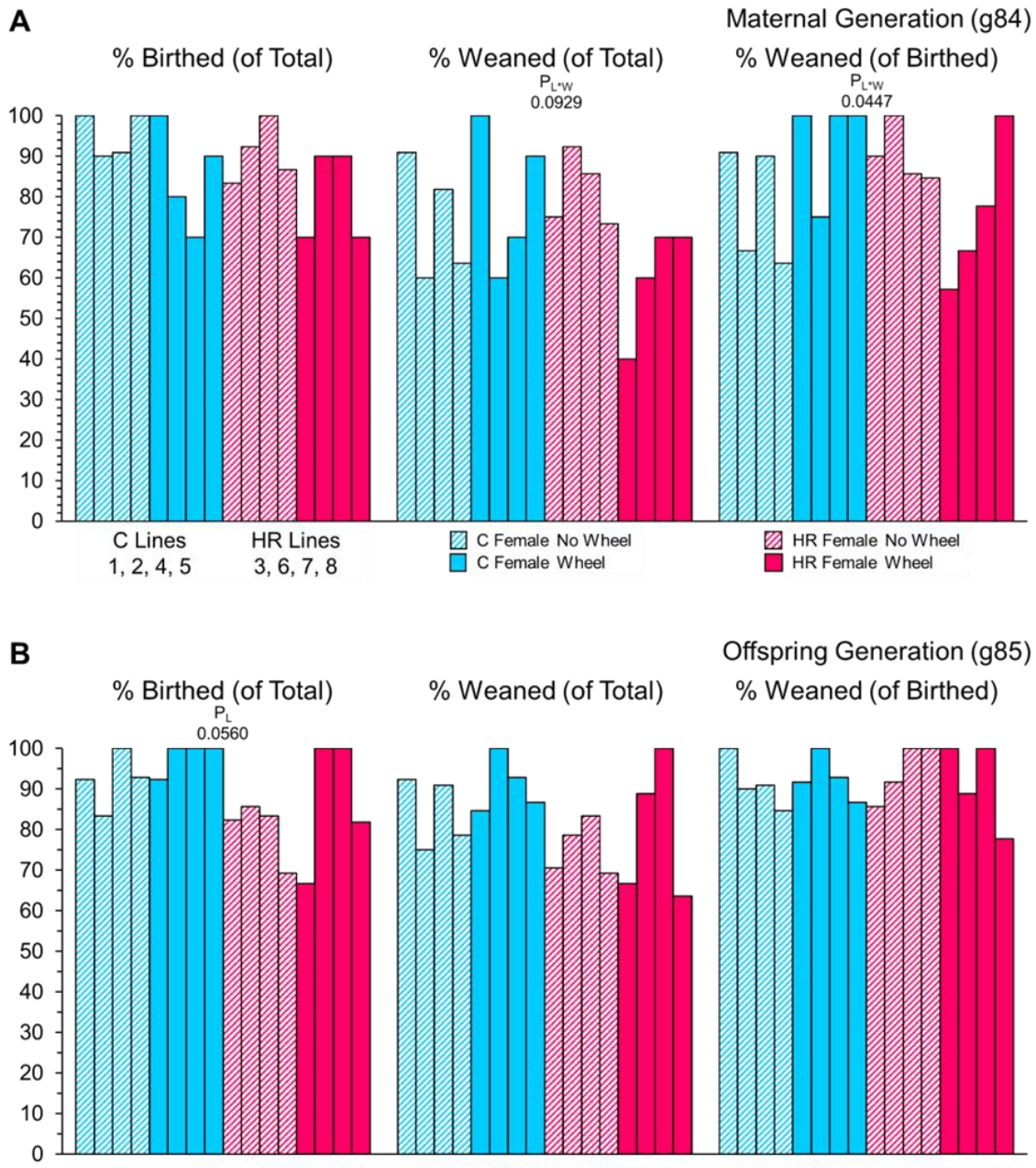


Figure 3.6. Continued.

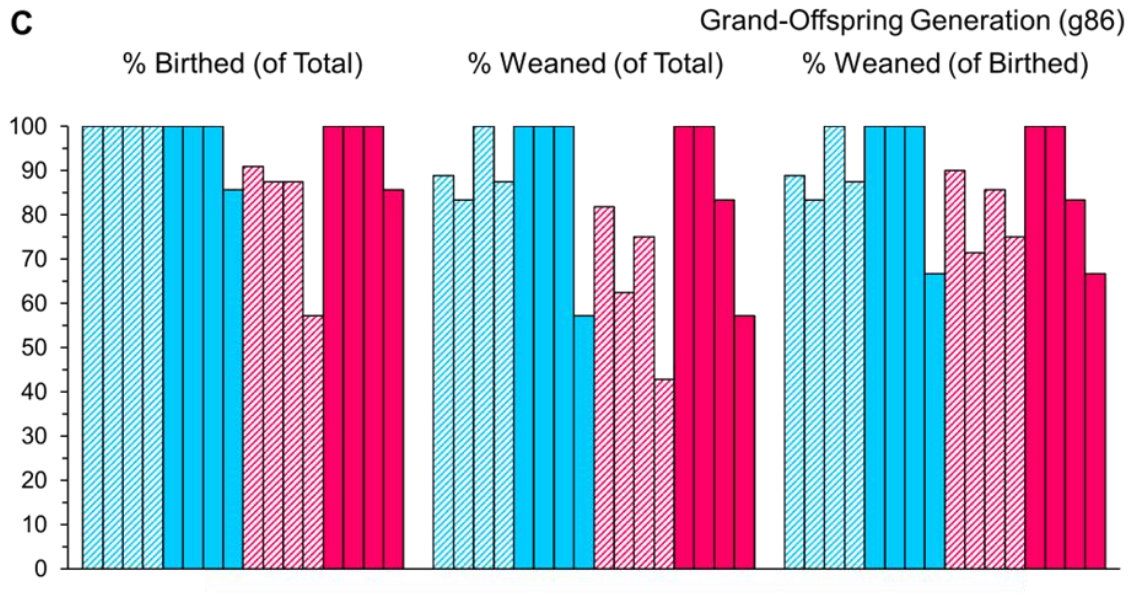
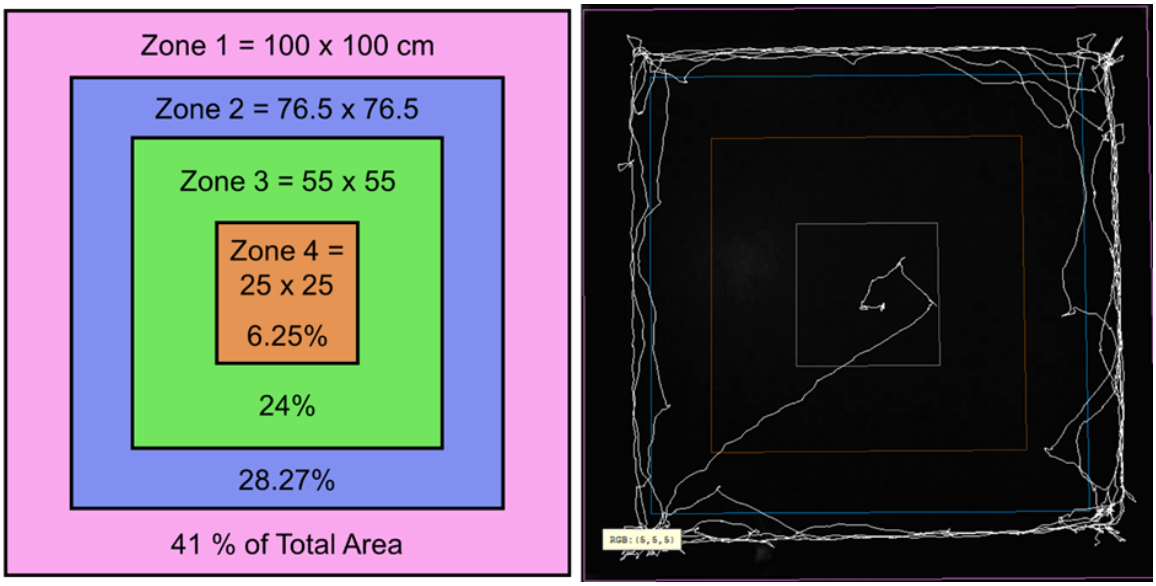


Figure 3.7. TopScan examples. (Left) Digital zones constructed within TopScan LITE video tracking software, with their corresponding linear dimensions and proportion of the total open-field arena. The open-field arena is 100 X 100-cm, and was constructed using 50-cm-high walls of black Trovicel plastic, held together with duct tape around the outside. The first zone was constructed and placed over the arena floor, such that the area of Zone 1 is equal to the surface area of the arena floor (i.e., 100 X 100-cm). The other zones were created as proportions of the first zone, corresponding to known distances of the physical arena. (Right) A representative trial from TopScan LITE after the open-field video has been analyzed. Trials were analyzed automatically, and TopScan LITE recorded the following variables in 1-minute bins for each of the 5 distinct zones: time before each zone is entered for the first time (or for the center, the time before the mouse leaves), the number of bouts (i.e., times a mouse entered a given zone), and, for each zone, the time spent, distance travelled, and average speed.



Concluding Remarks

In Chapter 1, I demonstrated that, despite a lack of statistical significance in most studies (Table 1.1, Figure 1.1), selection for voluntary wheel-running behavior resulted in an early divergence in body and relative heart mass effect sizes (Table 1.2, Figures 1.2D and 1.3D). Specifically, mice form the HR lines evolved to be smaller, but to have larger heart ventricles (with body mass as a covariate). In addition, the effect sizes for both traits plateau within the generational range wherein the selection limit for wheel running occurred in HR mice (Careau et al. 2013). Such correlated responses to selection indicate a genetic correlation between physical activity, body mass, and relative heart mass. In other words, some genes have pleiotropic effects on both wheel running and body mass or relative heart mass (linkage disequilibrium being a remote possibility). However, we can only speculate as to the functional basis of these relationships. Nonetheless, larger hearts evolving in response to selection for sustained, aerobically supported endurance exercise (i.e., wheel-running behavior) does make sense, and human marathoners are relatively small in size.

In the HR mouse experiment, few traits that have been repeatedly measured as extensively as body and relative heart mass. But some traits that have been sufficiently measured across the 100+ generations of selection, such as other organs related to exercise physiology (e.g., liver), or maximal oxygen consumption during exercise (VO_2max), that future meta-analyses can fruitfully examine their effect sizes. These traits have also varied in their statistical

significance across generations, which makes them good candidates for meta-analytic research. Similar to body and heart mass, underlying trends in the data may not be apparent on a case-by-case basis, or even by evaluation on the basis of statistical significance. Incorporating these traits into a broader meta-analysis would allow us to see whether the evolution of high activity behavior has coincided with broad changes in the effect sizes of multiple (potentially correlated) traits, or alternatively if few effect sizes have changed as wheel-running behavior evolved. Additionally, a broader meta-analysis could consider other potential moderating factors, such as mini-muscle status or season.

Of course, correlation still does not prove causation. For example, both body mass and relative heart mass could instead be functions of some other, currently undescribed mechanism. Causality could be established experimentally by selecting for one (or both) sub-organismal traits and recording any changes (or lack thereof) to wheel-running behavior (Garland Jr. 2003). Also, as described in Swallow et al. (1999), one could test whether one (or both) sub-organismal traits constrained the evolution of wheel running by selecting on both traits (e.g., body mass and wheel running) simultaneously. Subsequent selection experiments could provide much needed clarity on the evolutionary mechanisms and biological underpinnings of exercise behavior.

In Chapter 2, I tested two hypotheses related to the aerobic capacity model for the evolution of vertebrate energetics: (1) that selection for high levels of sustained aerobic physical activity required an increase in VO_2max, and (2)

that BMR also increased due to unspecified links with VO_2max. Although many have tested aspects of this model (e.g., see Książek et al. 2004; Gębczyński and Konarzewski 2009; Sadowska et al. 2015; Wone et al. 2015), none have directly tested its primary assertions. Despite HR mice running ~3-fold more revolutions per day than C mice, we only saw a modest change in VO_2max (a statistically significant 13.6% increase), a non-statistically significant 6.5% increase in BMR (Table 2.1, Figure 2.2), and no statistically significant changes in organ masses or hematocrit (Table 2.2, Figure 2.3).

The morphology-performance-behavior-fitness paradigm is a cornerstone of organismal biology (Arnold 1983; Garland Jr. and Kelly 2006; Storz et al. 2015). However, although behavior evolved rapidly (e.g., a 75% increase in wheel running by generation 10), various aspects of HR mouse performance have not differed in ways predicted from the MPBF paradigm. For example, sprint speed did not differ between HR and C mice at generation 22 (e.g., see Khan 2023), despite HR mice increasing daily running distance primarily via running speed. Additionally, measures of performance that now differ (Kolb et al. 2010; Hiramatsu et al. 2017; Cadney et al. 2022), such as VO_2max, have not always (e.g., statistically different in generation 10, see Swallow et al. 1998; but not statistically different in generation 22, see Khan 2023), particularly during periods where high running behavior was evolving (i.e., prior to the selection limit). Apparently, relatively minor changes in performance (and presumably underlying morphological and physiological traits) are capable of generating

disproportionate response at the level of behavior. The results found in Chapter 2 are also consistent with the "behavior evolves first" model, wherein behavior is highly adaptive, but does not always occur with corresponding morphological or physiological adaptations (e.g., see Blomberg et al. 2003; Huey et al. 2003; Rhodes and Kawecki 2009; Khan 2023). Although Chapter 2 focuses on aspects of exercise ability (e.g., VO_2max), selection for high voluntary wheel running has resulted in several changes to traits likely related to the motivation for exercise, notably to those involved in the reward pathway (Rhodes et al. 2001, 2005; Rhodes and Garland Jr. 2003; Belke and Garland Jr. 2007; Keeney et al. 2008, 2012; Saul et al. 2017; Thompson et al. 2017). Changes to motivation for exercise, as opposed to ability to exercise, could provide an explanation for large changes in behavior with modest changes to underlying metrics related to exercise physiology and locomotor performance.

In Chapter 3, I demonstrated that, when provided with a wheel, HR dams spend more time engaging in activity behaviors (including wheel running) and less time engaging in maternal care (Table 3.1: Maternal Behavior, Figure 3.5). This was ultimately detrimental to their offspring, as HR dams with wheels were the least successful in rearing offspring to weaning, despite similar levels of success in giving birth (Table 3.1: Offspring Weaning, Figure 3.6A). Surviving offspring also had delayed eye opening and reduced body mass at both weaning and sexual maturity, indicating they may have experienced stunted growth (Table 3.2, Figure 3.4B). These effects may be attributed to two potential sources: (1) in

utero effects of high maternal exercise, as HR dams ran more before birth than C dams (Table 3.1: Maternal Wheel Running, Figure 3.2) and/or (2) post-partum effects of decreased maternal care (described above). However, because we did not evaluate litter characteristics at birth, we could not determine which of the effects (or perhaps some combination of effects) had greater impact. Finally, despite these changes to offspring early-life phenotype, their body composition, activity behavior, and reproductive success as adults was unaffected by maternal wheel access (Table 3.2).

The Developmental Origins of Health and Disease (DOHaD) hypothesis (Gluckman and Hanson 2004; Barker 2007; Hanson and Gluckman 2008) is founded on two key postulates. First, offspring are able to adapt to conditions experienced in utero to produce a "thrifty phenotype;" that is, a phenotype that is somewhat pre-adapted to the expected external environment. Second, several maladies (such as cardiovascular disease, Type II diabetes, and obesity, e.g., see Hales and Barker 1992; Barker 1995, 2007; Hales et al. 2017) can be traced back to an evolutionary mismatch between the expected environment (which may be based on evolutionary history, in utero environmental effects, or maternal signaling) and the lived environment. As mentioned above (Chapter 3: Introduction), studies in humans indicate that adult body composition can be partially "programmed" by developmental experiences, based on the observation that high maternal weight gain often leads to heavy offspring (Vohr and McGarvey 1997; Hammami et al. 2001; Haugen et al. 2014) who are at higher

risk of obesity (Yu et al. 2011; Mamun et al. 2014; Chiavaroli et al. 2016; Godfrey et al. 2017), Type II diabetes (Wei et al. 2003; Johnsson et al. 2014) and cardiovascular disease (Allcock et al. 2009; Skilton et al. 2014; Gaillard 2015) as adults.

Interestingly, the growth trajectory of large babies, and the associated health effects later in life, has changed over the past 100 years. Babies classified as large at birth in the 1920s were found to have reduced morbidity and mortality compared with those of lower birth weight (Hales et al. 1991; Barker et al. 1993). This effect was present across the birth weight range, as there was a progressive increase in cardiovascular and metabolic risk with decreasing birth weight, even among those in the "normal" range. Additionally, large-at-birth infants were characterized as being long and lean (Gunderson and Abrams 1999; Gunderson 2009). Yet now, the association between birth weight and later-life disease risk appears to be U-shaped, and large at birth infants are characterized as being long and fat (Vohr and McGarvey 1997; Dabelea and Crume 2011). It is possible that the stark contrast in nutritional environment between the 1st and 2nd halves of the 20th century may explain the change in large at birth phenotype (Lee et al. 2022). That is, the 1st half of the 20th century was characterized by a relatively limited nutritional environment available to the wider population (e.g., due to the World Wars and Great Depression), whereas the 2nd half of the 20th century has been characterized by a relative nutritional excess (e.g., due to calorie dense foods and abundance of high-fructose corn

syrup). Thus, maternal energetic state has been identified as a potential target for early-life intervention (Birdsall et al. 2009; Alfaradhi and Ozanne 2011; Wasenius et al. 2017), with a focus on maternal weight and diet (Wu et al. 1998; Suzuki 1999; Wu and Suzuki 2006; Agha et al. 2014; Hiramatsu et al. 2017; Montalvo-Martínez et al. 2018). However, the last 100 years has also been characterized by another change -- to relative activity levels (Physical Activity Guidelines Advisory Committee 2008; Piercy et al. 2018; Du et al. 2019) -- and the role of maternal exercise on offspring is much less clear for this (Agha et al. 2014; Perales et al. 2016).

Generally, exercise is instrumental in preventing disease and promoting physical and mental health (Mokdad et al. 2004; Haskell et al. 2007; Mikkelsen et al. 2017). The CDC (Center for Disease Control, US) recommends ~30 minutes per day (~150 minutes per week) of moderate-to-vigorous intensity aerobic activity (e.g., a brisk walk, or jogging). However, most adults to not meet these recommended levels of activity (Physical Activity Guidelines Advisory Committee 2008; Du et al. 2019). The CDC recommends that pregnant individuals should maintain similar levels of activity throughout their pregnancy, if possible (Piercy et al. 2018). Yet, concerns regarding potential negative effects of exercise during pregnancy remain, and may deter women from exercise during critical periods of development (Walasik et al. 2020).

Maternal exercise during pregnancy has a number of clear, beneficial health outcomes for mothers (e.g., reduced risk of gestational diabetes and

improved post-partum recovery (Davies et al. 2003; Pivarnik et al. 2006; Borodulin et al. 2008)), but potential benefits to offspring health -- especially once offspring reach adulthood -- have been much less studied. Indeed, maternal exercise has only more recently received attention as a preventative strategy for offspring obesity (Birdsall et al. 2009; Wasenius et al. 2017). Studies in humans have shown that routine aerobic exercise during pregnancy can result in smaller offspring at birth (Hopkins et al. 2011). Several previous studies have examined the effects of maternal exercise on offspring characteristics in rodents (e.g., see reviews in Blaize et al. 2015; Kusuyama et al. 2020), demonstrating that maternal exercise often improves the metabolic health of offspring (e.g., see Carter et al. 2012; Laker et al. 2014; Blaize et al. 2015; Raipuria et al. 2015; Stanford et al. 2015), but has little effect on offspring body composition (e.g., see Kelly et al. 2015), and mixed results regarding offspring activity behavior (e.g., see Kelly et al. 2015; Eclarinal et al. 2016). Overall, our results indicate that, at least for these lines of mice, maternal exercise does not have long-lasting impacts on offspring spontaneous physical activity, voluntary physical activity, or body composition.

Going forward, I will be a postdoctoral research fellow at Chapman University in their Grand Challenges Initiative program. There, I will further develop my teaching abilities. I will also be working with Dr. Patricia Lopes to investigate possible neuroendocrine changes associated with parental care behavior in typically non-parental Japanese quail. I hope to take these skills, and

those I have gathered from dissertation research, to start my own lab, where I would study: (1) the genetic, environmental, and gene-by-environment interactions that influence maternal care in mammals, (2) the role maternal care behavior plays in the evolution of complex traits (e.g., exercise behavior), and (3) how hormones may act as the “tools” of evolutionary change (e.g., see Garland Jr. et al. 2016).

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