Effects of food restriction on voluntary wheel-running behavior and body mass in selectively bred High Runner lines of mice

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ABSTRACT

Food restriction can have profound effects on various aspects of behavior, physiology, and morphology. Such effects might be amplified in animals that are highly active, given that physical activity can represent a substantial fraction of the total daily energy budget. More specifically, some effects of food restriction could be associated with intrinsic, genetically based differences in the propensity or ability to perform physical activity. To address this possibility, we studied the effects of food restriction in four replicate lines of High Runner (HR) mice that have been selectively bred for high levels of voluntary wheel running. We hypothesized that HR mice would respond differently than mice from four non-selected Control (C) lines. Healthy adult females from generation 65 were housed individually with wheels and provided access to food and water *ad libitum* for experimental days 1-19 (Phase 1), which allowed mice to attain a plateau in daily running distances. *Ad libitum* food intake of each mouse was measured on days 20-22 (Phase 2). After this, each mouse experienced a 20% food restriction for 7 days (days 24-30; Phase 3), and then a 40% food restriction for 7 additional days (days 31-37; Phase 4). Mice were weighed on experimental days 1, 8, 9, 15, 20, and 23-37 and wheel-running activity was recorded continuously, in 1-minute bins, during the entire experiment. Repeated-measures ANOVA of daily wheel-running distance during Phases 2-4 indicated that HR mice always ran much more than C, with values being 3.29-fold higher during the *ad libitum* feeding trial, 3.58-fold higher with -20% food, and 3.06-fold higher with -40% food. Seven days of food restriction at -20% did not significantly reduce wheel-running distance of either HR (-5.8%, $P = 0.0773$) or C mice (-13.3%, $P = 0.2122$). With 40% restriction, HR mice showed a further decrease in daily wheel-running distance ($P = 0.0797$ vs. values at 20% restriction), whereas C mice did not ($P = 0.4068$ vs. values at 20% restriction) and recovered to levels similar to those on *ad libitum* food ($P = 0.3634$). For HR mice, daily running distances averaged 11.4% lower at -40% food versus baseline values ($P = 0.0086$), whereas for C mice no statistical difference existed (-4.8%, $P = 0.7004$). Repeated-measures ANOVA of body mass during Phases 2-4 indicated a highly significant effect of food restriction ($P = 0.0001$), but no significant effect of linetype ($P = 0.1764$) and no interaction ($P = 0.8524$). Both HR and C mice had a significant reduction in body mass only when food rations were reduced by 40% relative to *ad libitum* feeding, and even then the reductions averaged only -0.60 g for HR mice (-2.6%) and -0.49 g (-2.0%) for C mice. Overall, our results indicate a surprising insensitivity of body mass to food restriction in both high-activity (HR) and ordinary (C) mice, and also insensitivity of wheel running in the C lines of mice, thus calling for studies of compensatory mechanisms that allow this insensitivity.

Keywords:
Caloric restriction
Exercise
Genotype-by-environment interaction
Selection experiment
Spontaneous physical activity
Wheel running

1. Introduction

Many animals must deal with some degree of food deprivation or restriction at some point in their normal life cycle (Wang et al. 2006), and this also occurs in various experimental protocols in the laboratory (Rowland 2007). Numerous studies show that food restriction can have profound effects on various aspects of physiology, behavior, and anatomy. In rodents, these effects may vary in relation to the specific food restriction protocol (Hill et al. 1985; Varady 2011), species (Cornish and Mrosovsky 1965; Blank and Desjardins 1985), strain (Gelegen et al. 2006), age (Speakman and Mitchell 2011), and sex (Martin et al. 2007) (see also references in Sherwin 1998). Moreover, it might be expected that such effects would be amplified in animals that are highly active, given that (a) physical activity can represent a substantial fraction of the total daily energy budget and (b) the control of physical activity involves motivation and reward systems of the brain that overlap with those involved in the control of feeding behavior (Garland, Jr. et al. 2011b; Novak et al. 2012; Lightfoot et al. 2018; Ruiz-Tejada et al. 2022). Note that food restriction -- reducing the amount of food available on a daily basis -- is different from food deprivation, in which food is removed entirely for some period of time, such as 24 hours (Dill et al. 1978; Dietze et al. 2016). Rowland (2007) has reviewed some of the differences in behavioral and physiological responses of laboratory rodents to these different treatments, but simple generalities do not emerge, perhaps in large part because protocols have varied considerably.

In laboratory house mice, the cost of voluntary wheel-running activity can represent a substantial portion of the total energy budget (Swallow et al. 2001; Rezende et al. 2009). Also, as noted by Dewsbury (1980), use of running wheels as a measure of physical activity tends to exaggerate the effects of various experimental manipulations. Perhaps not surprisingly, food restriction has yielded variable results with respect to wheel-running activity of mice, with reported increases, no change, or decreases in distance traveled in response to the same food restriction protocol (-50%) in different strains of mice (Symons 1973). For example, Padovani et al. (2009) observed that the distance traveled decreased ~67% with 30% food restriction, in relation to an *ad libitum* food group, at the end of 6 weeks of the experiment. Moreover, Blank and Desjardins (1985) showed that the effect of 30% food restriction on distance traveled varied across the daily cycle, decreasing during the dark and increasing during the light period in wild-derived male house mice. However, this within-day effect was not observed in food-restricted deer mice (*Peromyscus maniculatus*), which ran less when restricted during both periods.

Within a given species, some of the differences among studies could be associated with intrinsic, genetically based variation in the propensity or ability to perform voluntary wheel running. For example, mouse strains with inherently high wheel-running levels during food restriction in "activity-based anorexia" protocols have more rapid loss of body mass (Pjetri et al. 2012). To further explore possible genetically based differences in the response to food deprivation, we studied four replicate lines of High Runner (HR) mice that have been selectively bred for voluntary wheel running (Swallow et al. 1998). Since reaching apparent selection limits around generation 17-25 (depending on line and sex: Careau et al. 2013), HR mice run voluntarily ~3-fold more revolutions per day than those from four non-selected Control (C) lines, and this differential has been approximately constant over more than 75 generations of continued selective breeding (Garland, Jr. et al. 2011a; Cadney et al. 2021; McNamara et al. 2022a; Schwartz et al. 2023). The nature of this apparent selection limit is presently unknown, but may be related to either motivational or physiological factors, or both (Claghorn et al. 2016). When housed without access to wheels, HR mice are more active than C mice in their home cages (Malisch et al. 2009; Copes et al. 2015), although HR mice are not more active than C mice in a 3-minute open-field test, which is considered a measure of exploratory behavior or reaction to a novel environment (Bronikowski et al. 2001; Careau et al. 2012; but see Cadney et al. 2021).

As compared with the C lines, HR mice have elevated endurance capacity (Meek et al. 2009) and maximal aerobic capacity (VO_2max) (Kolb et al. 2010; Cadney et al. 2021; Schwartz et al. 2023) during forced treadmill exercise, as well as various lower-level morphological and physiological traits that may affect endurance capacity (Rhodes et al. 2005; Swallow et al. 2009; Garland, Jr. et al. 2011b; Wallace and Garland, Jr. 2016). HR mice have reduced total body mass (Swallow et al. 1999) and body fat (Swallow et al. 2001; Vaanholt et al. 2008; Meek et al. 2010; Hiramatsu and Garland, Jr. 2018), which could affect their ability to contend with food restriction. HR mice also show alterations in their brain motivation and reward system, dopamine signaling, responses to endocannabinoid agonists and antagonists, and in the sizes of specific brain regions, including an enlarged hippocampus (Rhodes et al. 2005; Belke and Garland, Jr. 2007; Keeney et al. 2012; Kolb et al. 2013; Thompson et al. 2017; Schmill et al. 2023); again, these differences could affect their responses to food restriction (Belke and Pierce 2016; Liu and Kanoski 2018; Ruiz-Tejada et al. 2022). Finally, HR mice differ from C mice with regard to their fecal microbiota (McNamara et al. 2022b, 2022a), which could also affect responses to food restriction.

The effect of food restriction on HR mice has not been investigated. One reasonable expectation is that these unique mice would reduce the amount of wheel running to deal with periods of low food availability. However, in an experiment designed to address the effect of an increased amount of work needed to obtain food, the distance traveled between HR and C groups when they needed to work (run on wheels) for food did not differ (Vaanholt et al. 2007). Another reason to expect differences between HR and C mice is that the former show greater among-individual variation in daily wheel running distance, greater among-individual variation in plasticity of running, and greater unpredictability of running distances (Biro et al. 2018). Finally, male mice from the HR lines respond uniquely to feeding on a Western diet, with wheel running increasing dramatically, while C mice are unaffected (Meek et al. 2010, 2012; Acosta et al. 2017). In contrast, inbred C57BL/6J mice of both sexes reduce wheel running on a high-fat and high-sugar diet (Vellers et al. 2017). Therefore, the aim of this study was to determine the effects of food restriction on voluntary wheel-running behavior and body mass in HR mice, with the general hypothesis that they will respond differently than mice from the non-selected C lines. Such a result would set the stage for future studies aimed at uncovering the mechanisms underlying differential responses.

2. Methods

2.1. Experimental animals

Healthy adult female mice (N = 99) from generation 65 of an ongoing selection experiment for high voluntary wheel running were used (Swallow et al. 1998). We chose females for this initial study because they generally run more than males (e.g., Gelegen et al. 2007; Meek et al. 2009; Garland, Jr. et al. 2011a), thus providing more signal against which to detect potential reductions in wheel running.

The original progenitors of the colony were 224 outbred, genetically variable Hsd:ICR mice (Harlan Sprague Dawley, Indianapolis, Indiana, USA). After two generations of random mating, 8 closed lines were formed, with four selected for high voluntary wheel running (based on days 5 and 6 of a 6-day test) and four bred without regard to running (Swallow et al. 1998; Careau et al. 2013). Before the experiments described here, beginning at weaning, animals were housed in same-sex groups of up to four individuals in a standard cage (28 × 17 × 12 cm). Water and food [Harlan Teklad Rodent Diet (W) 8604] were available *ad libitum*. Complete information on the composition of this diet can be found at the Inotiv website (https://www.inotivco.com/rodent-traditional-natural-ingredient-diets). Room temperature was maintained at 22 to 24° C and photoperiod was 12:12, with lights on at 0700 Pacific time.

2.1. Protocols

As shown in **Figure 1**, adult female mice were housed individually with running wheels (1.12 m circumference, as used in the routine selection protocol (Swallow et al. 1998)), and provided access to food and water *ad libitum* for experimental days 1-19, which allowed the amount of daily wheel running to stabilize (e.g., see Swallow et al. 2001). Then, the mice were maintained in the same running-wheel cages for an additional three-day period (days 20-22) to measure baseline food intake (Koteja et al. 2003). After this, each mouse experienced a 20% food restriction (weighed and provided daily) for 7 days (days 24-30), and then a 40% food restriction for 7 additional days (days 31-37). Restriction amounts were determined individually for each mouse, using their baseline food consumption. Water was available *ad libitum* throughout the experiment.

2.3. Measurements

Mice were weighed on experimental days 1, 8, 9, 15, 20, and 23-37. Wheels were checked for freeness and mechanical or electrical malfunctions on a daily basis. Wheel-running activity was recorded continuously, in 1-minute bins, during the entire experiment. Animals were monitored daily, and any that appeared moribund or exhibited a loss of more than 30% body mass as compared with their mass at the start of food restriction (Gelegen et al. 2007) were intended to be removed from the experiment and returned to *ad libitum* food conditions, or euthanized. In practice, this did not occur.

2.4. Statistical analyses

Following numerous previous studies of these lines of mice (e.g., Kolb et al. 2010, 2013; Claghorn et al. 2016; Hiramatsu and Garland, Jr. 2018; Schmill et al. 2023), body mass, food consumption, and wheel running with *ad libitum* food were analyzed by mixed-models, with replicate line nested within linetype (HR vs. C). The degrees of freedom for testing the effect of linetype (i.e., the effect of past selective breeding) were always 1 and 6. Covariates were used as appropriate, e.g., age, body mass. In addition, several of the analyses used individual mice as repeated measures across days with an AR(1) covariance structure.

All analyses were performed in SAS Procedure Mixed, and data are presented as least squares means and associated standard errors. Mini-muscle status (Garland, Jr. et al. 2002; Kelly et al. 2013) was included as an additional cofactor in preliminary analyses, but as it did not appreciably affect the primary statistical results it was removed for simplicity in the final results reported here. In the present sample of mice, all of the individuals from HR line #3 were mini-muscle individuals, but only one from HR line #6 (see Syme et al. 2005). Statistical outliers (values for individual mice for particular days) were deleted when standardized residuals exceeded 3 in magnitude.

For analyses of body mass, food consumption, and wheel running, we also performed separate analyses (some of them repeated-measures) of the HR and C lines, treating line as a fixed effect within either selection group (Garland, Jr. et al. 2011a). We did this because differences among the replicate lines may be of interest in their own right (e.g., see Gammie et al. 2003; Garland, Jr. et al. 2011a; Kolb et al. 2013; Castro et al. 2022; Hillis and Garland, Jr. 2023; Schwartz et al. 2023) and because the vastly different starting values for wheel running (more than 3-fold greater in HR lines as compared with C lines) can obscure treatment effects in C lines due to scale effects.

3. Results

Figure 2 presents the results for daily wheel running and body mass as measured across the course of the experiment, separated by individual lines (4 HR and 4 C). The overall result is that wheel running was remarkably stable in the face of food restriction for mice from the non-selected Control lines, but declined significantly in mice from the selectively bred High Runner lines. Body mass of both C and HR mice showed modest decreases. Results are discussed in greater detail in the following sections, which can be interpreted in the context of Figure 2. Our emphasis is on differences in the average responses of the four HR lines versus the four C lines. However, as has been noted previously for a variety of traits, differences among the four replicate lines within each linetype do exist (e.g., see Garland, Jr. et al. 2011a; Castro et al. 2022; Hillis and Garland, Jr. 2023; Whitehead et al. 2023). Therefore, we also refer to analyses comparing lines within the two linetypes, and place the relevant materials in Supplemental Table 1.

3.1. Body mass and food consumption

During the *ad libitum* food consumption trial (experimental days 20-22), HR mice (Least Square Mean ± Standard Error: 24.99 ± 0.978 g) did not weigh significantly less ($P = 0.1453$) than C mice (27.32 ± 0.981 g). Age (mean = 86.3 days, range = 80-89) as a covariate did not have a significant effect ($P = 0.3698$). Significant differences in body mass also existed both among the four replicate HR lines and among the four replicate C lines (Supplemental Table 1).

Adjusting for age ($P = 0.8891$) and body mass ($P = 0.0183$) as covariates (overall mean body mass = 26.15 g), HR mice (6.19 ± 0.198 g/day) ate approximately 23% more ($P = 0.0073$) than C mice (5.04 ± 0.201 g/day)($N = 100$ mice). When the total amount of wheel running (revolutions) during the three-day food trial was added as an additional covariate, it was a highly significant positive predictor of food intake ($P = 0.0008$) and the difference in food intake between HR (5.84 ± 0.226 g/day) and C mice (5.45 ± 0.231 g/day) was reduced and became statistically non-significant ($P = 0.3229$) (body mass $P = 0.0493$)($N = 99$ because one mouse with a faulty wheel was removed). Hence, the greater food intake by HR female mice, versus their C counterparts, when both are housed with wheel access, can be mostly explained by the greater levels of voluntary exercise by HR mice, as has been reported previously (Copes et al. 2015; but see Hiramatsu and Garland, Jr. 2018). In these analyses, differences among the replicate C lines were not statistically significant ($P = 0.6258$), but those among the HR lines were ($P = 0.0217$) (Supplemental Table 1).

3.2. Wheel running during the initial 6 and 19 days of exposure

During the first six days of wheel access, as is used routinely to choose breeders in the selection experiment, wheel running was always higher in HR than in C mice (P < 0.0001), increased across days (P < 0.0001) for both HR and C, but also showed a strong day * linetype interaction (P < 0.0001) (**Figure 3A**). Inspection of the graph indicates that running by HR mice increased more rapidly across days 3-6 than for C mice. For example, the difference in average wheel revolutions run per day increased from 2,247 on days 1-2 to 3,646 on days 5-6 for C mice (1.6-fold) but from 6,450 to 13,309 for HR mice (2.1-fold). Analyses of the Control lines alone indicated strong line and day effects, but no day * line interaction (Supplemental Table 1). In contrast, analyses of the HR lines indicated no line effect (P = 0.9838), but a strong day effect and a marginal day * line interaction (P = 0.0503).

Considering the components of wheel running during the first six days of wheel access, the number of 1-minute intervals with any running (**Figure 3B**) was always higher in HR than in C mice (P = 0.0465), increased across days (P < 0.0001) for both HR and C, with no significant interaction (P = 0.1623). The mean running speed (**Figure 3C**) was always higher in HR than in C mice (P < 0.0001), increased across days (P < 0.0001) for both HR and C, but also showed a strong day * linetype interaction (P < 0.0001). Results were similar for the maximum revolutions attained in any 1-minute interval on a given day ($P_{linetype} < 0.0001$, $P_{day} < 0.0001$, $P_{interaction} = 0.0003$) (**Figure 3D**).

Considering days 7-19, daily wheel-running distance continued to increase (**Figure 4A**), but in a less monotonic fashion, and reached plateaus at least by day 16 for both HR and C mice, with highly significant effects of linetype, day, and their interaction (all P << 0.0001). Averaged over days 15-19, daily revolutions run were 4,643 for C mice and 17,473 for HR mice, a ratio of 3.8. Analyses of the Control lines alone indicated strong line and day effects, and a marginal day * line interaction P = 0.0710) (Supplemental Table 1). Analyses of the HR lines indicated a strong day effect (P << 0.0001) and a day * line interaction (P = 0.0067), but no overall line effect (P = 0.5675).

3.3. Wheel running during food restriction

The repeated-measures ANOVA of HR and C lines combined indicated that daily distance run was strongly affected by linetype (P < 0.0001) and marginally affected by food restriction (P = 0.0632), with no significant interaction between linetype and food restriction status (P = 0.1399) (total N = 99 mice and 1,661 observations). Based on these combined analyses, and as shown in **Figure 5A**, HR mice always ran much more than C, with values being 3.29-fold higher during the *ad libitum* feeding trial, 3.58-fold higher with 20% food restriction, and 3.06-fold higher with 40% restriction.

Based on the combined analyses, seven days of food restriction at 20% did not significantly reduce wheel running of either HR (-5.8%, P = 0.0773) or C mice (-13.3%, P = 0.2122) (**Figure 5A**). With 40% restriction, HR mice showed a further reduction (P = 0.0797 vs. values at 20% restriction, -11.4% versus baseline values), whereas C mice slightly increased running (P = 0.4068 vs. values at 20% restriction), resulting in a value that was only 4.8% below those on *ad libitum* food. Comparing the four C lines indicated differences among them (P = 0.0101) and the strong effect of food restriction (P = 0.00224), with no significant restriction * line interaction (P = 0.1692) (Supplemental Table 1). Results were similar when comparing the four HR lines (P values were 0.0270 for line, 0.0380 for level of food restriction, and 0.2629 for their interaction).

Considering the components of wheel running, the repeated-measures ANOVA of HR and C lines combined indicated that the duration of daily running was affected by both linetype ($P = 0.0404$) and food restriction status ($P = 0.0078$), with no interaction ($P = 0.2535$), and an overall pattern similar to that for daily distance run, except that the difference between HR and C mice is much less (**Figure 5B**). For average running speed (**Figure 5C**), results were similar to those for distance run, with a highly significant effect of linetype ($P < 0.0001$), an effect of food restriction ($P = 0.0231$), but also a significant interaction between linetype and food restriction status ($P = 0.0354$. Finally, results for maximum running speed were similar to those for average speed ($P_{linetype} < 0.0001$, $P_{restriction} = 0.0822$, $P_{interaction} = 0.0072$) (**Figure 5D**).

For the number of intervals run, the repeated-measures ANOVA comparing the C lines indicated strong line ($P < 0.0001$) and food restriction ($P < 0.0001$), with a marginal line * restriction interaction $P = 0.0537$) (Supplemental Table 1). Similar results held for the HR lines ($P_{line} < 0.0001$, $P_{restriction} = 0.0112$, $P_{interaction} = 0.1049$).

Average running speed of the C mice was unaffected by line ($P = 0.6785$), restriction ($P = 0.2944$) or their interaction ($P = 0.9021$), whereas, in contrast, HR mice showed effects of both line ($P = 0.0267$) and restriction status ($P = 0.0026$) ($P_{interaction} = 0.3295$). Maximum running speed was affected only by line in both C mice ($P = 0.0004$) and the HR mice ($P = 0.0027$) (Supplemental Table 1).

3.4. Body mass during food restriction

For the repeated-measures ANOVA of HR and C lines combined, we compared body masses measured on days 20 and 23 (*ad libitum* food), 24-30 (-20% food), and 31-37 (-40% food)(total N = 99 mice and 1,574 observations). The effect of food restriction was highly significant ($P = 0.0001$), with a non-significant ($P = 0.1764$) tendency for HR mice to be smaller (-8%, LSMeans of 24.99 ± 0.90 for C mice and 23.03 ± 0.90 for HR mice), and no interaction ($P = 0.8524$). **Figure 6** shows that both HR and C mice had a significant reduction in body mass only when food rations were reduced by 40% relative to *ad libitum* feeding. However, even at 40% food reduction, the decrease in body mass was only -0.49 grams for C mice and -0.61 for HR mice, which is only 2.0% and 2.6%, respectively, of the body mass prior to food reduction.

Comparing the four C lines indicated differences among them ($P = 0.0015$) and the effect of food restriction ($P = 0.0008$), with no restriction * line interaction ($P = 0.5721$) (Supplemental Table 1). Results were similar for the HR lines ($P_{line} = 0.0007$, $P_{restriction} < 0.0001$, $P_{interaction} = 0.8082$).

4. Discussion

The main results of this study were as follows. First, both HR and C female mice maintained body mass (no statistically significant reduction) on a 20% food restriction regimen that lasted for seven days (**Figure 6**), without a statistically significant decrease in daily wheel-running distance (**Figure 5A**). Second, with a 40% food reduction for an additional week, C mice increased running distance back closer to those measured under *ad libitum* feeding, while experiencing a significant decrease in body mass that averaged only -2.0% relative to *ad libitum*-fed values. Third, High Runner mice on 40% food restriction showed a further decline in running distance (**Figure 5A**) that became significantly lower than on *ad libitum* food, and did then show a statistically significant reduction in body mass (**Figure 6**), but the magnitude was only -2.6% compared with *ad libitum* feeding. Examination of the components of daily wheel-running distance (duration and mean speed) indicate that both were affected by food restriction, but in ways that differed between the HR and C lines

(**Figure 5B and 5C**). Specifically, 40% food restriction decreased both duration and speed of running in the HR mice, but only decreased duration in the C mice.

Many previous studies with rodents show that food restriction can have a range of effects, including on behavior and activity in the cage and/or wheel. Various factors combined may alter the effect of restriction, including the duration and intensity (% reduction), age, sex, and species (e.g., see Symons 1973; Hill et al. 1985; Padovani et al. 2009; Varady 2011). Several studies use different combinations of these factors, which can make it difficult to compare results. One large study of inbred C57BL/6 mice found that even 40% calorie restriction lasting for 80 days had modest effects on wheel running (Mitchell et al. 2016). Further complicating matters may be the presence of expected or hypothesized psychological changes, some of which follow a stress response, that may interact with some effects of food restriction. For example, our mice were housed individually for wheel-running measurements, and social isolation has sometimes been shown to increase circulating levels of corticosterone in mice (Takatsu-Coleman et al. 2013), which is routinely taken as one indicator of a response to stressful conditions. However, other studies have not observed social isolaton to increase corticosterone levels in mice (Misslin et al. 1982). An Unpredictable Chronic Mild Stress (UCMS) protocol can reduce voluntary running wheel in mice (DeVallance et al. 2017), and, conversely, access to wheels can affect circulating corticosterone levels in various ways (Girard and Garland, Jr. 2002; Droste et al. 2006), both acutely and chroncially, and decrease fecal corticosterone metabolite levels (Gurfein et al. 2012).

Another factor that may potentiate the physiological effects of calorie restriction is combination with exercise (Huffman 2010). This combination has been used in the treatment of obesity. In obese laboratory mice, for example, combined calorie restriction with wheel exercise caused greater reduction of adiposity when compared to a group that only experienced calorie restriction (Patterson and Levin 2007).

In the present study, we did not observe a statistical increase in wheel-running activity at either 20% or 40% food restriction, which is unlike what happens in activity-based anorexia models with mice and rats (Exner et al. 2000; Gutiérrez et al. 2002; Hebebrand et al. 2003; Siegfried et al. 2003; Overton and Williams 2004). In addition, the drop in body mass we observed even with 40% calorie restriction (~2-3%) is much smaller than in activity-based anorexia protocols administered to mice (Gelegen et al. 2007; Pjetri et al. 2012). In those models, the animals have free access to the wheel, but with *ad libitum* access to food for only a short period of time, typically only once each day (e.g., Gelegen et al. 2006, 2007; Pjetri et al. 2012). These differences in the protocols do not allow us to compare results directly. However, the increased activity in animals subjected to an activity-based anorexia protocol has been linked to increases in circulating corticosterone concentrations (Duclos et al. 2009). Thus, it is interesting to note that mice from the HR lines typically have baseline circulating corticosterone concentrations that are roughly twice as high as for Control mice (Malisch et al. 2007, 2008), perhaps suggesting that further increases were not possible for HR mice [although acute increases in response to restraint stress are possible: (Malisch et al. 2016)]. In addition, HR mice are closer to a biological limit in terms of wheel running, at least with the standard chow diet used in the present study (e.g., see Meek et al. 2009). Perhaps HR mice are also less sensitive to changes related to corticosterone concentrations or other factors that may have contributed to the increased activity observed in mice from the non-selected C lines. Finally, the low circulating leptin levels of HR mice, as compared with C lines (Girard et al. 2007;

Meek et al. 2012), could play a role (Garland, Jr. et al. 2011b, 2016; Mitchell et al. 2016; Ruiz-Tejada et al. 2022).

Our results differ from a study of two lines of rats, one selectively bred for high (HCR) and the other low (LCR) intrinsic aerobic capacity during forced treadmill running (Koch and Britton 2001). HCR rats also run more on wheels and weigh less than LCR rats (Waters et al. 2008; Swallow et al. 2010). With 50% calorie restriction for three weeks, female high-capacity rats lost more weight than low-capacity rats, and high-capacity rats also had a greater decrease in home-cage physical activity levels (though levels remained higher than low-capacity rats) (Smyers et al. 2015). As noted previously, differences in our results may be due to the different species, amount and length of food restriction, and selection paradigm.

In summary, our results indicate an unexpected degree of insensitivity in both body mass and voluntary wheel-running behavior to food restriction, which calls for further studies of compensatory mechanisms that allow this insensitivity in these and possibly some other strains of laboratory house mice (Symons 1973; Pjetri et al. 2012; Vaanholt et al. 2012; Jensen et al. 2013; Mitchell et al. 2016). One might wonder if the magnitude and duration of food restriction used here were sufficient to induce effects on the phenotypes measured. However, previous studies have shown effects within a span of 7 days. For example, Symons (1973) examined body mass and daily wheel running in four inbred strains under a 50% food restriction protocol. All four inbred strains dropped in body mass, with a greater decrease seen with each of the six successive trial days, and some differences among the strains were apparent. All four strains showed effects of food restriction on daily wheel running, although the effects differed among strains. Importantly, mice from two of the four strains died after two days at -50% food, which is one of the reasons we used the less extreme -20% and then -40% levels of food restriction. In another example, mice from an inbred strain showed reductions in body mass and increases in wheel running after three days at -30% of ad lib food (Tezenas du Montcel et al. 2023). Thus, we are confident that the degree of food restriction we imposed was of sufficient magnitude that effects on body mass and/or wheel running would have been expected.

As noted above, differences in responses in the present study may be influenced by known differences in corticosterone, leptin, and/or endocannabinoid levels in HR compared with C mice. Also of considerable interest would be studies of sex differences in responses to food restriction (Dietze et al. 2016), especially given that, in both the HR and C mice used here, the sexes differ in daily wheel-running distance, body composition, endocannabinoid physiology, reproductive physiology (obviously), and many other aspects of the phenotype (e.g., see Rezende et al. 2009; Hiramatsu and Garland, Jr. 2018; Schmill et al. 2022; Khan et al. 2024). Moreover, as the food restriction part of our study lasted only two weeks, it would be interesting to see if the increase in wheel running by C mice in response to 40% food restriction would be able to be supported for longer periods of time (e.g., see Vaanholt et al. 2015; Tezenas du Montcel et al. 2023).

We also note that our results are relevant to the point that ad lib feeding of laboratory rodents often leads to overfed animals that may be "metabolically morbid" (Martin et al. 2010). As pointed out by those authors, housing mice or rats with less than ad lib food is not too difficult, and doing so (perhaps in conjunction with access to an exercise wheel, e.g., see Booth and Lees 2006) may improve the translational relevance of results.

In closing, we suggest that our results may have translational relevance for the regulation of body weight in human beings. Although countless studies of humans examine relationships among diet, exercise, body composition, and energetics

(Drenowatz 2015; Lightfoot et al. 2018; Careau et al. 2021), few have tested specifically for the effects of caloric restriction on voluntary exercise (Rowland 2016). Studies of humans conducted inside respiratory chambers usually find that spontaneous physical activity (SPA) does not change during calorie restriction (see summary and references in Martin et al. 2011), but SPA and voluntary exercise are very different aspects of physical activity in both humans and rodents (Garland, Jr. et al. 2011b; Copes et al. 2015; Rowland 2016; Acosta et al. 2017). In a study of short-term overfeeding (3 days), obesity-prone individuals significantly decreased the amount of time spent walking (Schmidt et al. 2012) (see also Levine et al. 2008). In studies of free-living people, three randomized trials examining the effect of calorie restriction (-20% to -30%) in nonobese adults of both sexes (Martin et al. 2011) found reductions in activity energy expenditure over 3-12 months, but accelerometry provided little evidence for reductions in moderate, hard or very hard intensity activity (how much of this involved voluntary exercise is not stated), although other studies have reported variable results (Martin et al. 2011; Drenowatz 2015). Our results seem consistent with those of Martin et al. in that caloric restriction did not cause a substantial reduction in voluntary exercise. This may be good news with respect to dieting to lose weight because it suggests we may not need to worry that levels of voluntary exercise will decline as part of the behavioral and physiological (energetic) compensatory mechanisms that sometimes occur in response to dieting.

Data availability statement

Data are available upon reasonable request from the authors.

Conflict of interest statement

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi: xx

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Figures and Legends

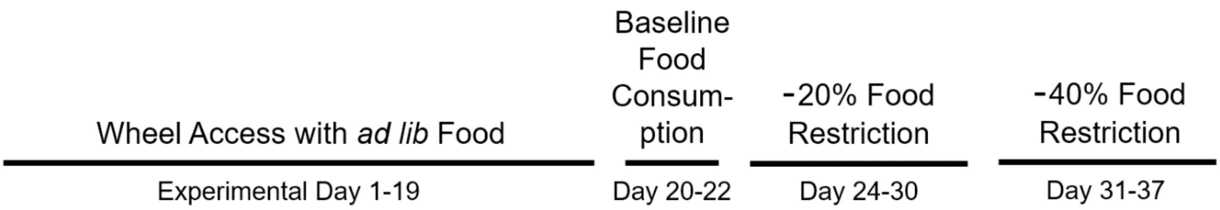

Figure 1. Experimental timeline.

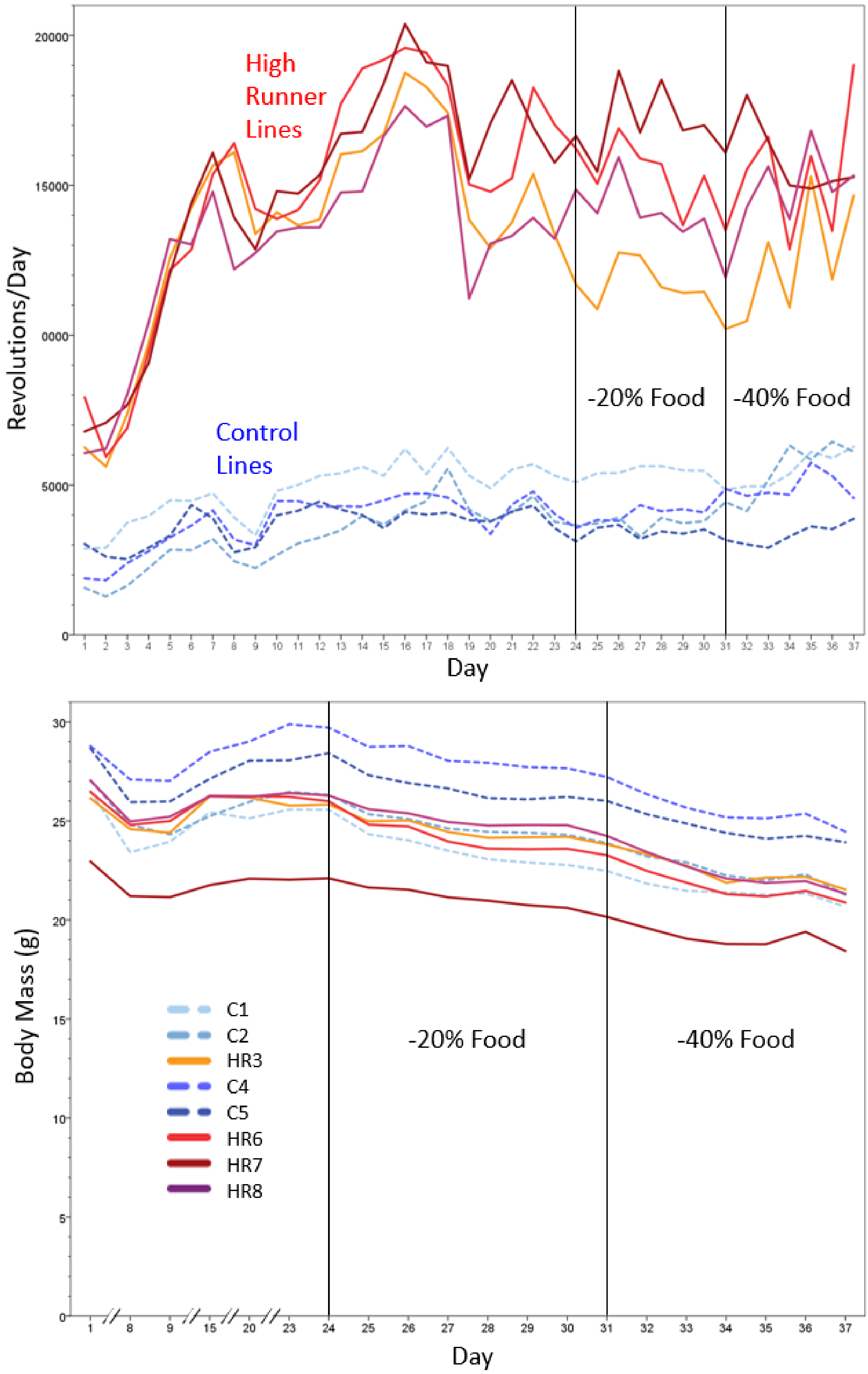

Figure 2. Overall results for wheel running measured daily and for body mass measured on days 1, 8, 9, 15, 20, and 23-37. Values are simple means for each of the four High Runner (lines 3,6,7,8) and four non-selected Control lines (1,2,4,5) of mice. Note that the horizontal axis scales differ for the two panels.

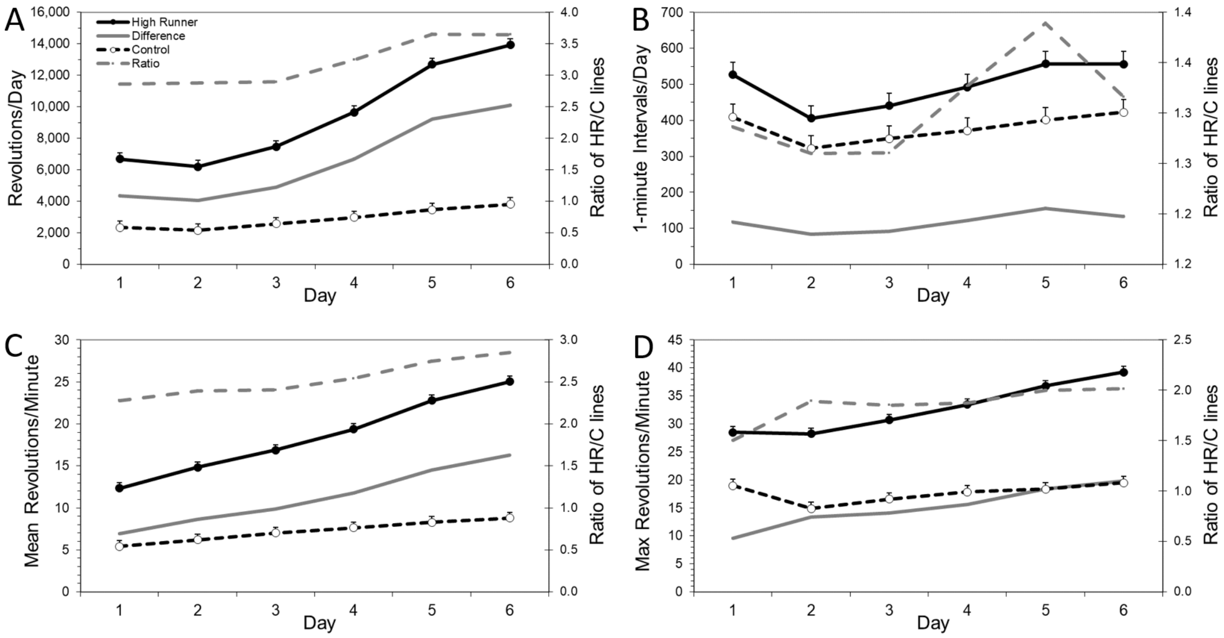

Figure 3. A) Average wheel running (revolutions/day) of female mice from four replicate High Runner (HR) lines (solid black line with closed circles) and four non-selected Control lines (dashed black line with open circles) during the first six days of wheel access, as is used to pick breeders during the routine selective breeding protocol (see text). The statistical interaction between day and linetype is highly significant ($P < 0.0001$). Inspection of the graph indicates that running by HR mice increases more rapidly across days 3-6 than for C mice. Values are Least Squares Means ± Standard Errors from SAS Procedure Mixed. Total N = 587 measurements from 99 mice. B) Number of 1-minute intervals per day with any wheel revolutions. C) Average running speed computed as revolutions/intervals on an individual mouse and day basis (RPM). D) Highest running speed observed during any minute of a day.

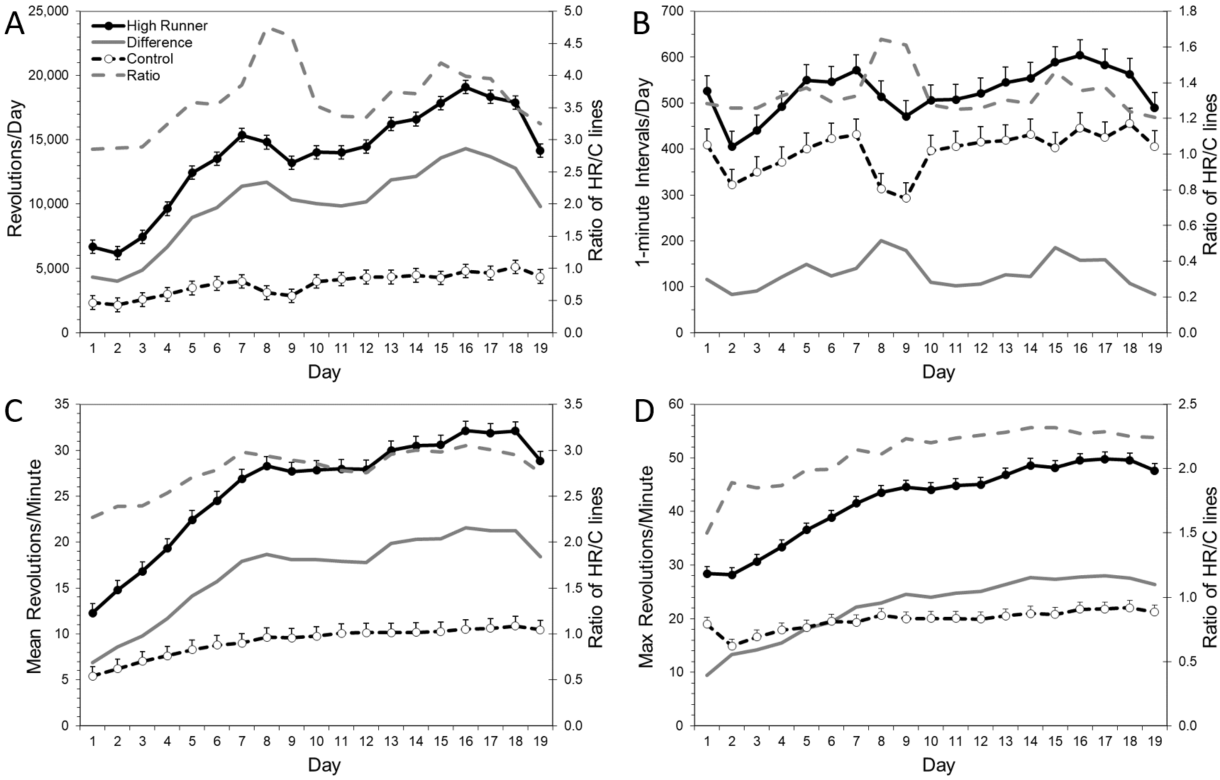

Figure 4. A) Average wheel running (revolutions/day) of female mice from four replicate High Runner (HR) lines (solid black line with closed circles) and four non-selected Control lines (dashed black line with open circles) during the first 19 days of wheel access (including data for the first six days, as shown in Figure 3). The statistical interaction between day and linetype is highly significant ($P < 0.0001$), indicating different longitudinal trajectories. Values are Least Squares Means $\pm$ Standard Errors from SAS Procedure Mixed. Total N = 1,862 measurements from 99 mice. B) Number of 1-minute intervals per day with any wheel revolutions. C) Average running speed computed as revolutions/intervals on an individual mouse and day basis (RPM). D) Highest running speed observed during any minute of a day.

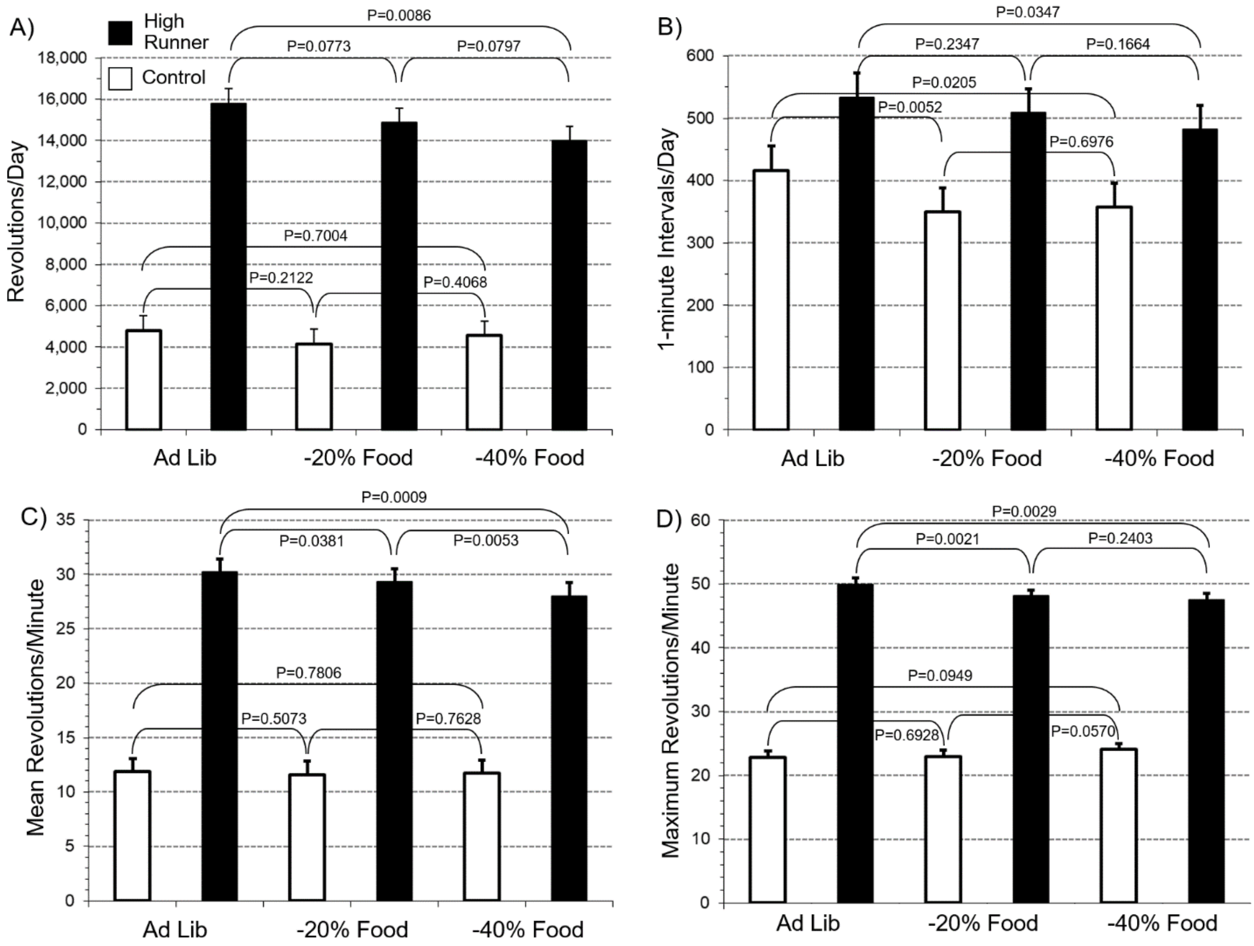

Figure 5. A) Average wheel running (revolutions/day) of female mice from four replicate High Runner (HR) lines and four non-selected Control lines during three days of *ad libitum* food (days 20-22 of the overall experiment), seven days of food restriction at -20%, and seven days of food restriction at -40%. See text for full description of statistical results. Shown on the figure are P values for differences of least squares means from SAS Procedure Mixed from combined analyses of the HR and C lines of mice. Bars are Least Squares Means $\pm$ Standard Errors. Total N = 1,661 measurements from 99 mice. B) Number of 1-minute intervals per day with any wheel revolutions. C) Average running speed computed as revolutions/intervals on an individual mouse and day basis (RPM). D) Highest running speed observed during any minute of a day.

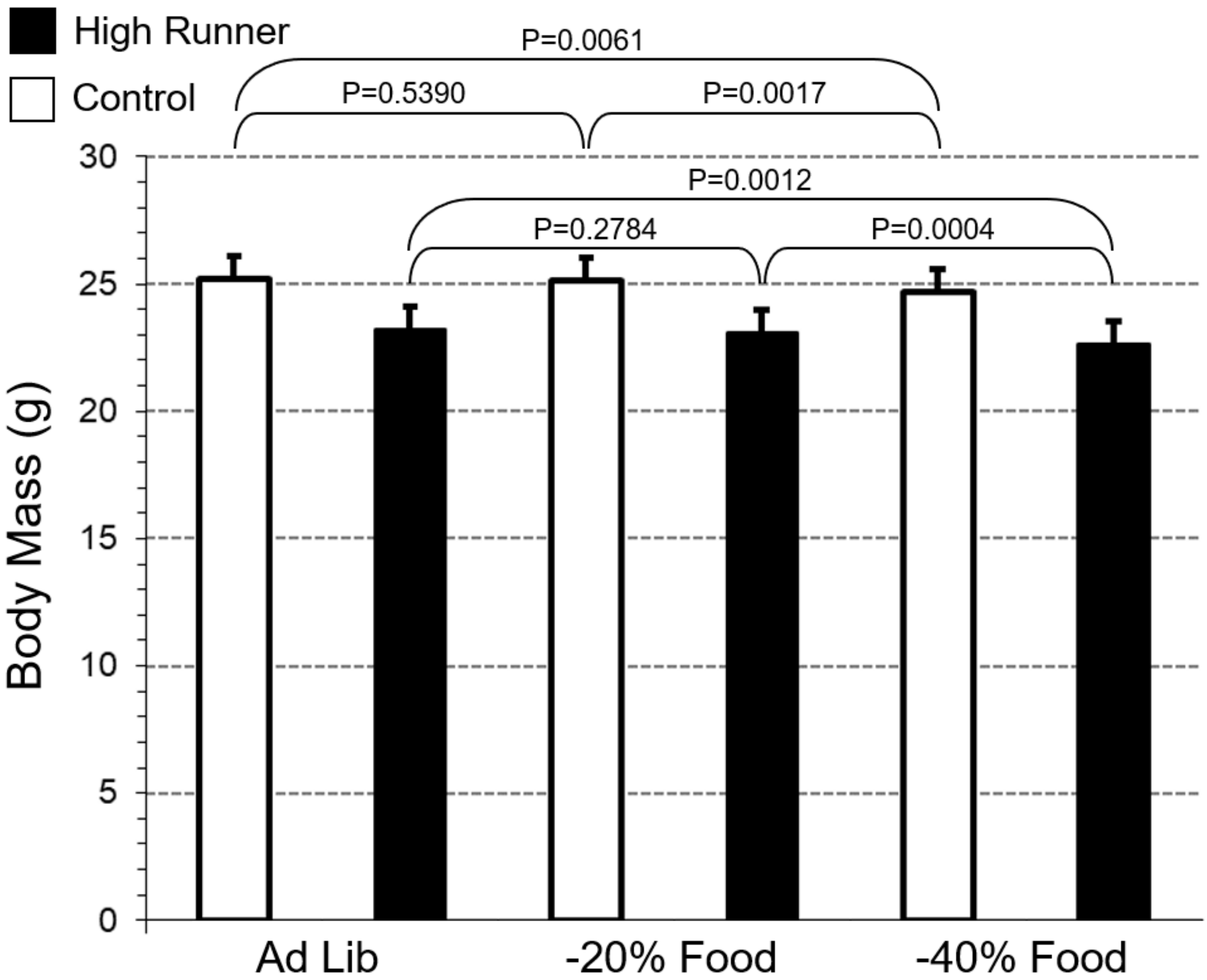

Figure 6. Average body mass (grams) of female mice from four replicate High Runner (HR) lines and four non-selected Control lines during three days of *ad libitum* food, seven days of food restriction at -20%, and seven days of food restriction at -40%. A repeated-measures ANOVA of all mice combined indicated the effect of food restriction was highly significant (P = 0.0001), with a non-significant (P = 0.1764) tendency for HR mice to be smaller (-8%, LSMeans of 24.99 $\pm$ 0.90 for C mice and 23.03 $\pm$ 0.90 for HR mice), and no interaction (P = 0.8524). Shown on the figure are P values for differences of least squares means from SAS Procedure Mixed. Both HR and C lines of mice showed a significant reduction in body mass only when food rations were reduced by 40% relative to *ad libitum* feeding. Values are Least Squares Means $\pm$ Standard Errors. Total N = 1,590 measurements from 99 mice.