Maternal exposure to Western diet affects adult body composition and voluntary wheel running in a genotype-specific manner in mice

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ABSTRACT

Some human diseases, including obesity, Type II diabetes, and numerous cancers, are thought to be influenced by environments experienced in early life, including *in utero*. Maternal diet during the perinatal period may be especially important for adult offspring energy balance, potentially affecting both body composition and physical activity. This effect may be mediated by the genetic background of individuals, including, for example, potential "protective" mechanisms for individuals with inherently high levels of physical activity or high basal metabolic rates. To examine some of the genetic and environmental factors that influence adult activity levels, we used an ongoing selection experiment with 4 replicate lines of mice bred for high voluntary wheel running (HR) and 4 replicate, non-selected control lines (C). Dams (half HR and half C) were fed a "Western" diet (WD, high in fat and sucrose) or a standard diet (SD) from 2 weeks prior to mating until their pups could feed on solid food (14 days of age). We analyzed dam and litter characteristics from birth to weaning, and offspring mass and physical activity into adulthood. One male offspring from each litter received additional metabolic and behavioral tests. Maternal WD caused pups to eat solid food significantly earlier for C litters, but not for HR litters (interaction of maternal environment and genotype). With dam mass as a covariate, mean pup mass was increased by maternal WD but litter size was unaffected. HR dams had larger litters and tended to have smaller pups than C dams. Home-cage activity of juvenile focal males was increased by maternal WD. Juvenile lean mass, fat mass, and fat percent were also increased by maternal WD, but food consumption (with body mass as a covariate) was unaffected (measured only for focal males). Behavior in an elevated plus maze, often used to indicate anxiety, was unaffected by maternal WD. Maximal aerobic capacity (VO$_2$max) was also unaffected by maternal WD, but HR had higher VO$_2$max than C mice. Adult lean, fat, and total body masses were significantly increased by maternal WD, with greater increase for fat than for lean mass. Overall, no aspect of adult wheel running (total distance, duration, average running speed, maximum speed) or home-cage activity was statistically affected by maternal WD. However, analysis of the 8 individual lines revealed that maternal WD significantly increased wheel running in one of the 4 HR lines. On average, all groups lost fat mass after 6 days of voluntary wheel running, but the absolute amount lost was greater for mice with maternal WD resulting in no effect of maternal WD on absolute or % body fat after wheel access. All groups gained lean and total body mass during wheel access, regardless of maternal WD or linetype. Measured after wheel access, circulating leptin, adiponectin, and corticosterone concentrations were unaffected by maternal WD and did not differ between HR and C mice. With body mass as a covariate, heart ventricle mass was increased by maternal WD in both HR and C mice, but fat pads, liver, spleen, and brain masses were unaffected. As found previously, HR mice had larger brains than C mice. Body mass of grand-offspring was unaffected by grand-maternal WD, but grand-offspring wheel running was significantly increased for one HR line and decreased for another HR line by grand-maternal WD. In summary, maternal Western diet had long-lasting and general effects on offspring adult morphology, but effects on adult behavior were limited and contingent on sex and genetic background.

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1. Introduction

The obesity epidemic has been expanding at an alarming rate. Poor diet and lack of physical activity are generally viewed as key components contributing to the increase, but recent research highlights the importance of several other factors, including environmental experiences in early life [1]. Specifically, maternal overnutrition during the perinatal stage (gestation + lactation) has been associated with increased risk for childhood obesity, cardiovascular diseases, and other ailments, with effects often lasting into adulthood. Changes to epigenetic regulation of these traits might be passed on to further generations, compounding the epidemic.

Rodent models have reported that high-fat diet induces offspring leptin insensitivity and altered hypothalamic and hippocampal function through direct and epigenetic effects ([2–4], review in [5]). Maternal high-fat diets have been reported to decrease offspring birth mass in mice [6] and rats [7,8], but increase offspring mass (with increase in fat and lean mass) from weaning into adulthood [7,9,10]. Studies of laboratory rats found that dams fed a high-fat diet have greater fat content in their milk after the first week of lactation (no difference in the first week) and increased milk production [11,12].

Maternal high-fat diet alters the glucocorticoid pathway of the dam, affecting offspring *in utero* and in later development in mice [13]. Offspring of dams receiving a high-fat diet may have higher corticosterone levels as adults [14]. Maternal high-fat diets may also lead to increased adult leptin [9,15,16] and decreased adiponectin [15]. Maternal overnutrition during this stage has also been reported to alter neurobiological processes, including dietary preferences, reward signaling, learning, and memory (e.g., [17–20]). Changes in maternal care have also been reported to impact stress responses of offspring as juveniles to adults. Maternal high-fat diet increased anxiety-related behaviors of offspring in an elevated plus maze in mice [2] and rats [21]. In another rat study, maternal high-fat diet decreased adolescent offspring anxiety, but increased adult offspring anxiety [13], suggesting potential differences in the effect of maternal diet at various offspring ages.

High levels of physical activity reduce risks for various diseases and promote mental health and physical fitness. Levels of physical activity are likely influenced by maternal diet. In mice, a cross-fostering study found decreased spontaneous physical activity (SPA) and increased obesity in female pups of genetically obese (A^{yy}/a) dams, even when they were fostered from birth to lean (a/a) dams [22]. Similarly, cross-fostering mice to smaller litters at birth increased body mass and adiposity and decreased physical activity and energy expenditure, apparently related to sex-specific alterations in hypothalamic DNA methylation and gene expression [23]. Another study of mice found that maternal Western diet decreased adult offspring activity in their home cage [24], though a similar study of rats observed no significant effect [25]. Maternal diet with added sunflower oil actually elevated SPA of offspring at 20 and > 65 days of age in one rat study [26]. Another study found that adult offspring of mice given high carbohydrate diets were hyperactive in their home cage [24,27]. Voluntary exercise was decreased by maternal WD in a study of mice [28]. Clearly, maternal diet (and potentially other environmental factors, [29,30]) can affect offspring physical activity and related traits. (At least one other study found effects of maternal over-nutrition on measures of locomotor behavior that have little to do with habitual spontaneous physical activity in home cages or voluntary exercise on wheels [31].)

Effects of maternal diet are likely dependent on the genetic background and associated behavioral and physiological traits of both the mother and her offspring. For example, individuals with inherently high levels of physical activity or high basal metabolic rates might experience some degree of "protection" from the adverse effects of maternal Western diet. This possibility could be addressed in various ways, such as through comparisons of strains of rodents that vary in activity levels (e.g., [32]). Here, we used the high runner (HR) mouse lines that have been bred for increased voluntary wheel running for 70 + generations and compared them with non-selected control lines. HR lines run 2.5–3 × times more revolutions per day when given wheel access (review in [33]) and are more active in their home-cage without wheels [34]. The HR lines have also been reported to increase adult wheel running when given Western diet from weaning through adulthood, indicating changes in energy balance as compared with the C lines [35]. HR lines show changes in other relevant lower-level traits, including increased heart mass, increased VO$_2$max, increased circulating corticosterone and adiponectin concentrations, but reduced leptin levels, and an altered brain reward system [36–39].

We tested the overarching hypothesis that the early-life environment can affect both spontaneous physical activity and voluntary exercise of adults, but that these effects would be different for HR and control lines of mice. We hypothesized that the genetic predisposition for high voluntary wheel running in HR mice would be protective against negative consequences of a maternal high-fat, high-sucrose diet. We further predicted that these effects could be mediated by changes in epigenetic regulation of genes as described above, in which case we could see the effects in the grand-offspring of dams fed WD. The replication of selected HR lines (N = 4) and non-selected C lines (N = 4) in this selection experiment helps to mimic the polygenic nature of human population differences in such complex traits as physical activity.

2. Materials and methods

All experiments and methods were approved by the Institutional Animal Use and Care Committee of the University of California, Riverside.

2.1.1. Experimental animals

Mice used for this experiment were from generation 73 of an ongoing, long-term artificial selection experiment that breeds for high voluntary wheel running ([33], for reviews, see [40]). A base population of 224 outbred individuals from the Hsd:ICR strain of house mice was bred randomly for 2 generations, then separated into 8 closed lines, each starting with 10 breeding pairs. The 8 lines were randomly designated into 4 lines bred for high voluntary wheel running (HR: lab designated as lines 3, 6, 7, 8) and 4 control lines bred without regard to wheel running (C: lab designated as 1, 2, 4, 5). Each generation, 6–8 week old mice from HR and C lines are individually housed for 6 days in cages attached to a Wahman-type activity wheel (1.12 m circumference, 35.7 cm diameter, 10 cm wide wire mesh running surface) with a recording device to count revolutions of the wheel in 1-min intervals for the duration of the experiment. In the HR lines, the highest running female and male from each family are chosen as breeders for the next generation based on their wheel running on days 5 and 6. Breeders are chosen randomly for C lines. All families are represented in the breeders to the next generation (termed "within-family selection") and sibling pairs are disallowed. Room temperatures are maintained at approximately 22 °C, with lights on at 0700 for a 12:12 photoperiod, and water and standard diet (SD: Teklad Rodent Diet W-8604, 14% kJ from fat, 54% kJ from carbohydrates, and 32% kJ from protein, no added sugars [less than ~9% naturally occurring sugars by weight, mostly from grains]) are available *ad libitum*.

2.1.2. Litter characteristics

In the present study, we manipulated the diet of dams (from generation 72 of the selection experiment) from 2 weeks before mating until pups were observed attempting to feed on solid food (~14 days after birth). All dams were given wheel access as young adults prior to diet manipulation as a normal selection generation. Dams were 14–16 weeks old when they gave birth. 100 dams (50 HR, 50 C) were
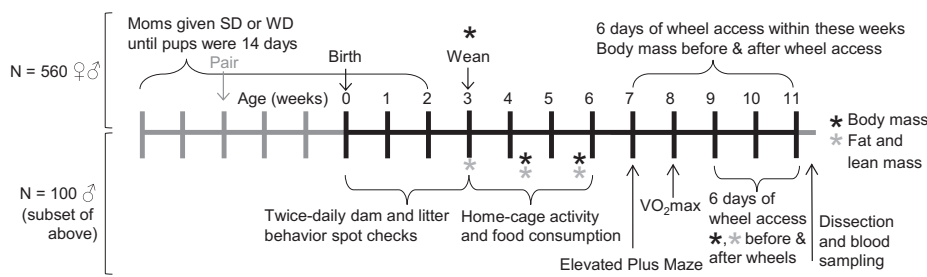
Fig. 1. Timeline of experimental design. Above the line are procedures given to all mice (N = 560), which include maternal diet manipulation (SD = standard diet, WD = Western diet), weaning at 3 weeks and 6 days of wheel access in adulthood. Below the line are procedures for 100 "focal" families and one male offspring from each of those families, which includes twice-daily behavior checks during the first 3 weeks, monitoring of home-cage activity and food consumption, behavioral testing on an elevated plus maze, and maximal aerobic capacity (VO₂max). Each tick mark represents one week and asterisks indicate when mice were weighed (in black) and measured for fat and lean composition (in grey).

fed a "Western" diet (WD: Harlan Teklad TD.88137, 42% kJ from fat, 42.7% kJ from carbohydrates, 15.2% kJ from protein, 34.1% added sucrose by weight) and another 100 dams (50 HR, 50 C) stayed on SD. The source of fat in WD was anhydrous milk fat, the source of protein was casein, and the sources of carbohydrates were sucrose and corn-starch (34.1 and 15.0 g/100 g, respectively). In addition, the high-fat diet contained 0.15% cholesterol.

Starting from birth and continuing until the pups were weaned at 3 weeks of age, 100 families (50 WD, 50 SD) were observed twice daily for developmental markers of the pups (i.e., first day for eye opening, moving, and feeding on solid food). Each cage was observed with a quick look ("spot check"). When pups were 15 days old, dams and litters were all switched to SD. Pups were weaned at 21 days of age and weighed; 2 males and 2 females were saved for C litters and up to 5 males and 5 females were saved for HR litters. One additional male pup from 50 WD dams and 50 SD dams (half C and HR in each group) were considered focal mice and received various additional tests (Fig. 1).

2.2. Body mass and body composition

All mice were weighed at weaning (3 weeks old) and before and after 6 days of wheel access (7–11 weeks old).

Focal males were additionally weighed at ∼4.5 weeks and ∼5.5 weeks of age. Focal male body composition was also measured by non-invasive quantitative magnetic resonance (EchoMRI-100; Echo Medical Systems LLC, Houston, Texas, USA). The body composition scanner independently calculated fat mass, lean mass, and water mass in grams. Fat mass was analyzed as such and as a percentage of total body mass.

Change in body mass after wheel access was calculated as an absolute change (post-exercise minus pre-exercise mass) and as percent change:

$$\frac{\text{post-exercise mass} - \text{pre-exercise mass}}{\text{pre-exercise mass}} \times 100$$

Similarly, change in lean mass was calculated as an absolute change and using the equation above. Change in fat mass was calculated as an absolute change in grams, change in percent fat mass, and using the equation above with fat mass in grams and percent fat mass.

2.3. Food consumption

For focal males, juvenile food consumption of SD (grams/day) was measured from 3 to 6 weeks of age. Food hoppers were weighed and any obvious shredding or wasting of food was noted. Food consumption was calculated as the absolute change in grams from 3 to 4.5 weeks of age and again from 4.5 to 6 weeks of age.

2.4. Maximal aerobic capacity (VO₂max)

VO₂max was measured during forced exercise in a 900 ml enclosed

wheel metabolic chamber as described previously [41,42]. Each trial lasted 5 min and each mouse was tested twice, with a rest day in between. Trials started by placing a mouse in the wheel chamber and manually spinning the wheel, slowly increasing the spinning speed over the trial. Air was pumped into the wheel at 2000 ml per min. A sub-sample of air (150 ml) was pumped out, ran through Drierite and soda lime to remove moisture and CO₂, and then the volume of O₂ was measured in an oxygen analyzer (S-3A Applied Electrochemistry INC. Sunnyvale, CA). Outputs from the instruments were digitized by an analog-to-digital converter (ADAM-4017 data Acquisition Module) and recorded every second on a computer using LabHelper software (Warthog Systems, www.warthog.ucr.edu). The highest 1-min interval of oxygen consumption in either trial was used to measure VO₂max per mouse.

Trials were graded for quality according to how cooperative the mouse was in running on the wheel (1 through 5) and how tired it looked after the trial (1, 2 or 3). Highly cooperative trials indicated that the mouse kept up with the wheel speed until a clear limit in O₂ consumption had been reached. Tiredness was graded by how many seconds (< 1, 1–5, or > 5 s) elapsed after the trial before the mouse was able to walk, groom, or engage in exploratory activities. The wheel apparatus for measuring VO₂max was chosen over the more traditional treadmill-based test because they obtain equivalent values and the wheel apparatus closely mimics the behavior for which HR mice have been bred [43].

2.5. Behavior in the elevated plus maze

As adults, focal males were tested for their behavior in an elevated plus maze. The maze consists of a plus-sign shaped platform, 1 m off the ground, with two exposed and two enclosed arms (length: 100 cm, width: 9 cm) joined to a central square platform (9 cm × 9 cm). Each trial lasted 5 min and the percentage of time a mouse spends in the enclosed arms is often used as a measure of anxiety [44]. Behavior in the maze was obtained at 1000 h–1400 h (3–7 h after lights on) in a lit room, recorded with HD Webcam C525 (Logitech International S.A., Lausanne, Switzerland) and analyzed using TopScan LITE software (Clever Sys, Inc., Reston, Virginia, USA). The surface of the maze was cleaned before each trial and mice were placed in the center square at the beginning of the trial.

2.6. Spontaneous physical activity

From weaning to 6 weeks of age, focal males were housed individually and monitored daily for home-cage activity using passive infrared motion-detector sensors [45,46]. A computer with custom Activity Recording Software (developed by Dr. Mark A. Chappell, UC Riverside) measured activity per 1-min intervals for 23 h [39]. Activity in home cages was also measured during wheel testing (described in Section 2.1.1).

2.7. Organ masses and plasma hormone concentrations

Focal males were sacrificed by decapitation 6–16 days post wheel access (counterbalanced by diet and linetype). Analyses of organs and hormones used as a covariate the number of days between the end of wheel access and sacrifice. Blood samples and various organs or tissues were dissected and weighed: posterior subcutaneous fat pads, caudal portions of the abdominal pelvic fat pad [47], heart ventricles, livers, spleens, and brains. Heparinized blood samples were spun at 13,000 RPM for 12 min and collected plasma was stored at −20 °C.

Plasma leptin was measured using a Crystal Chem Enzyme-linked Immunosorbent Assay (ELISA) kit (Mouse Leptin Assay Catalog #90030), without dilution and measured in duplicate in 96-well plates. Absorbances were read at 450 nm in an EPOCH2 microplate reader, using GEN5 2.07 reading software (microplate and reading software: BioTek Instruments, Inc., Winooski, VT, USA) and compared with a standard curve generated individually for each plate. Plasma adiponectin was measured similarly with an AssayPro ELISA kit (Mouse Adiponectin ACRP30 Catalog #EMA 2500-1), diluted 400-fold and measured in duplicate. Plasma corticosterone was measured similarly with an Arbor Assays ELISA kit (Corticosterone EIA kit Catalog #K014-H1), diluted 150-fold and measured in duplicate.

Leptin and adiponectin hormone concentrations were analyzed with covariates of fat percent of body mass.

2.8. Grand-offspring characteristics

To measure the effects of grand-maternal diet, males and females whose dams had the same diet (SD or WD) were paired to breed following the same protocol as the normal selection experiment. That is, for each HR line, we chose the highest-running male and female from each family and paired them to the highest runners from other families, but in this case only to mice whose mothers had the same diet. In each C line, we chose breeders without regard to their wheel running, but also paired based on maternal diet and disallowed mating between siblings. Then, the offspring of these pairings (i.e., grand-offspring of dams fed SD or WD) were given 6 days of wheel access as adults and weighed before and after.

2.9. Statistical analyses

All analyses were performed using the Mixed Procedure in SAS 9.1.3 (SAS Institute, Cary, NC, USA) to apply analysis of covariance models with Type III tests of fixed effects and REML estimation. Linetype (HR or C) and maternal diet were fixed effects; replicate lines were nested within linetype as a random effect. Effects of linetype, maternal diet, and their interaction were tested relative to the variance among replicate lines, and degrees of freedom were always 1 and 6 for these effects. The foregoing description applies to both the 100 focal males and the ~560 male and female offspring for which we obtained data on body mass at weaning, before and after adult wheel access, as well as adult wheel running for 6 days (Fig. 1). For the latter set of mice, we also used dam as a random effect nested within linetype to allow for possible litter effects.

Covariates depended on the trait being analyzed and included age, body mass, wheel freeness (inverse measure of rotational resistance), home cage sensor sensitivity [46], total wheel running (revolutions), and/or time from end of wheel access to sacrifice. In the results when we refer to traits being "adjusted for" by various variables, we mean that these variables were used as covariates in ANCOVA. Dependent variables were transformed as necessary to improve normality of residuals. All P values are 2-tailed unless otherwise indicated.

Body mass and voluntary wheel running were further analyzed by line to measure differences among replicate populations. Although HR lines experienced the same directional selection, each replicate line (and sexes within lines) differed somewhat in the rate and magnitude of

response to selection [48], as well as other phenotypes ([33], e.g., [40,49]). Thus, the replicate HR lines have had "multiple solutions" [50] and may be expected to differ in response to manipulation of maternal diet. When the interaction between diet and line was statistically significant, we checked the P value for differences of least-squares means for effect of diet on each line (SAS Procedure Mixed).

Mini-muscle status was also included as a cofactor in some analyses for focal males. Mini-muscle is a simple Mendelian genetic trait [51,52] that causes ~50% reduced hindlimb muscle mass in two HR lines (all mice in line 3 and a subset in line 6). Mini-muscle status was determined for focal males at the end of the study by inspection of triceps surae muscle mass regressed on body mass. All 10 focal males in line 3 (4 SD and 6 WD) and 3 of the 15 focal males in line 6 (1 SD and 2 WD) were mini-muscle individuals. Among several other phenotypic effects observed in adult mice, mini-muscle individuals were reported previously to have enlarged internal organs (e.g., [36,53,54]) and an elevated cost of transport during voluntary wheel running [55].

3. Results

Western diet had a variety of effects on the behavior and body mass of both dams and their offspring, and some of these effects were specific to mice from replicate lines (genotype-by-environment interaction).

3.1. Litter characteristics

All analyses of litter characteristics used age of the dam as a covariate. Analyses of pup behaviors were performed with and without litter size as a covariate. Litter size was not significant ($P > 0.05$) when used as a covariate, so the results without litter size are presented here (Fig. 2A, B, and C; see Online Supplemental Table 1 for results when litter size was included). The timing of the first occurrences for any pup of a litter to move on their own or open their eyes were not significantly affected by maternal WD and did not differ between HR and C lines (Fig. 2A and B, respectively). The first sighting for a pup to eat solid food was earlier for C litters with maternal WD (Diet-by-linetype interaction; Fig. 2C).

Adjusted for dam age, dam mass at weaning was not significantly affected by maternal WD and did not differ between HR and C (Online Supplemental Table 1). Analyses of litter size, total litter mass, and mean litter mass are shown in (Fig. 2D, E, and F). Adjusted for dam mass and age, the number of pups per litter at weaning was not significantly affected by maternal WD but was larger for HR than C dams (Fig. 2D). Total litter mass was increased by maternal WD but did not differ between HR and C families (Fig. 2E). Mean pup mass was increased by maternal WD and tended to be lower for HR than C (Fig. 2F). Adjusting for dam age, time from pairing to birthing pups was not affected by maternal WD and did not differ between HR and C mice (Online Supplemental Table 1). Sex ratio of pups (measured as number of female pups divided by total number of pups) was also not affected by maternal WD and did not differ between HR and C mice (Online Supplemental Table 1).

3.2. Body mass

Body mass at weaning was increased by maternal Western diet for both female and male offspring (Fig. 3A and B), and HR males were smaller than C males (Fig. 3B). Juvenile male mass was also increased by maternal WD at ~5 weeks of age and ~6 weeks of age (Fig. 4). Females were not weighed as juveniles.

Body mass at the start of wheel testing (~10 weeks of age) was increased for both females and males by maternal WD (Fig. 3C and D), and HR males were significantly smaller than C males (Fig. 3D). All groups significantly gained body mass during wheel access, regardless of maternal WD or linetype. However, the amount of increase in body mass after 6 days of wheel access was not affected by maternal WD or
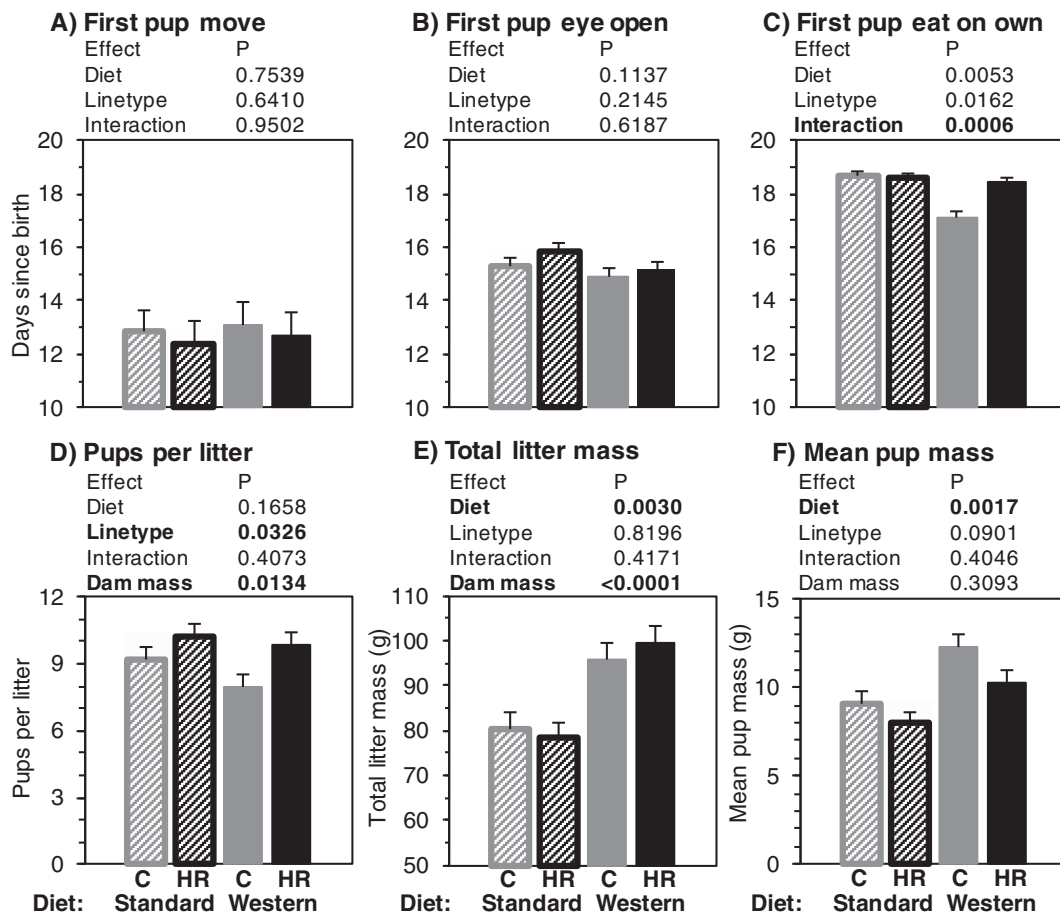
Fig. 2. Litter characteristics. A–C: for each litter, the first day that developmental markers were observed for at least one pup. D–F: number of pups per litter and total and mean pup mass at weaning (3 weeks after birth), adjusted for dam mass (grand mean = 29.34 g, standard deviation = 3.38 g). N = 25 per group. Bars represent least-squares means (LSM) + standard error (SE). Striped bars indicate maternal standard diet, solid bars indicate maternal Western diet, grey bars are control mice, and black bars are high runner (HR) mice. Results of statistical analyses (fixed effects) are shown above each graph. All analyses included dam age as a covariate (P values not shown).

linetype (Online Supplemental Table 1). Analyses of body mass in relation to replicate lines are presented in (Supplemental Fig. 4).

3.3. Focal male body composition

Both lean and fat masses were increased by maternal WD at all measurements from weaning to adulthood (Fig. 4). The increase for fat mass was greater than for lean mass in terms of both absolute and relative (% increase) values (results not shown). All groups gained lean mass during wheel access (all $P < 0.05$ except HR whose mothers had SD, for which $P = 0.0841$). However, the amount of increase in lean mass after 6 days of wheel access was not affected by maternal WD or linetype (Online Supplemental Table 1). Absolute and percent change in lean mass after 6 days of wheel access was not affected by maternal WD and did not differ between HR and C mice (Online Supplemental Table 1).

Regardless of maternal diet or linetype, mice lost fat after 6 days of wheel access. The decrease in absolute fat mass was significantly greater for mice whose mothers had WD ($P = 0.0340$), though percent change in fat percentage was not affected by maternal WD and did not differ between HR and C mice (Online Supplemental Table 1).

3.4. Food consumption

Food consumption of SD was measured for juvenile focal males from 3 to 6 weeks of age. Using body mass as a covariate, food consumption was not affected by maternal WD or linetype, with no interaction (Online Supplemental Table 1).

3.5. Maximal aerobic capacity (VO₂max)

For focal male mice, mass- and age-adjusted VO₂max was unaffected by maternal WD, but HR had higher VO₂max than C mice (Fig. 5). Mini-muscle individuals had elevated VO₂max.

3.6. Behavior in the elevated plus maze

Total duration in the maze varied slightly (range = 293–314 s), so trial duration was used as a covariate in all analyses. Mice spent 54.4% ± 13.6% (mean ± standard deviation) of the 5-min test in the closed arms of the maze, compared with 23.7% ± 12.5% in the open arms and 21.7% ± 9.2% in the center. They moved on average 15.9 m total (range: 4.00–20.66 m). Distance moved in each section was 11.2 ± 3.2 m in the closed arms, 2.6 ± 2.1 m in the open arms, and 2.1 ± 0.9 m in the center square.

We analyzed measures putatively related to anxiety, including percent entries into open arms and percent of time spent in open arms [44], as well as number of fecal pellets and urine pools at the end of the trial. We also analyzed, for each zone of the elevated plus maze (closed arms, open arms, and the center): latency to enter from the start of the test, number of entries, time spent, distance moved, and velocity. None of the above measurements of behavior were significantly affected by maternal WD or linetype, with no interaction and no effect of mini-muscle status (Online Supplemental Table 1 presents a subset of these analyses).
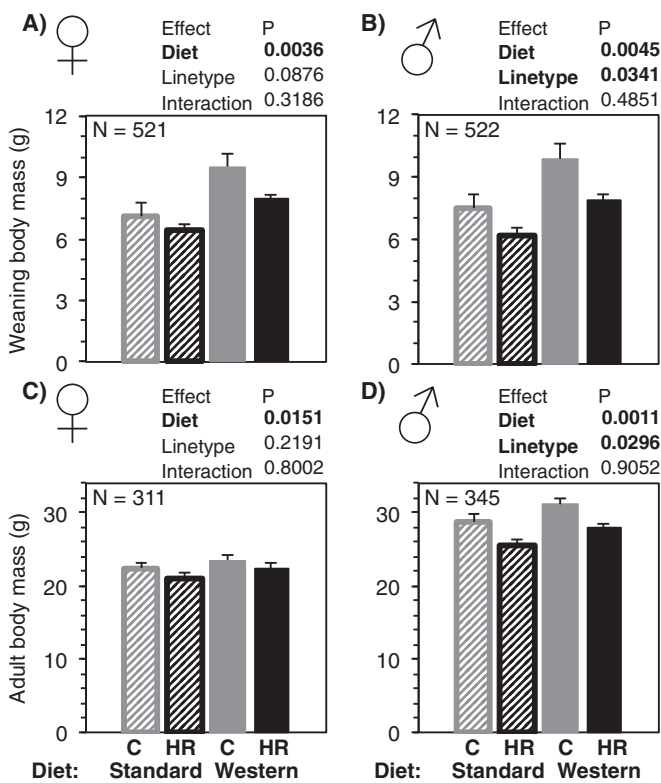
Fig. 3. Body mass at weaning (3 weeks of age) and as adults (just prior to wheel testing, 7–11 weeks of age) of C and HR mice given maternal SD or WD, with separate analyses for females and males. Bars are age-adjusted LSM + SE, and N were approximately equal for each group. Striped bars indicate maternal standard diet, solid bars indicate maternal Western diet, grey bars are control mice, and black bars are high runner (HR) mice. Results of statistical analyses (fixed effects) are shown above each graph. All analyses included age as a covariate (P values not shown).

3.7. Spontaneous physical activity

Maternal WD significantly increased home-cage activity of focal HR and C males at 3–4 and at 4–5 weeks of age, but by 5–6 weeks of age the effect was no longer statistically significant (Fig. 6). At 5–6 weeks of age, HR mice tended to be somewhat more active than C mice, but this effect was not statistically significant. Adding body mass to the models did not change statistical results in any important way (results not shown).

Adult focal male home-cage activity (measured during the 6 days of wheel access) was statistically unaffected by maternal WD, linetype, or their interaction. Additional analyses with amount of wheel running on days 5 and 6 as covariates did not change results (Online Supplemental Table 1).

3.8. Voluntary exercise

Wheel running of adult females was 3-fold higher in HR than C and 3.1-fold higher in adult male HR than C (Fig. 7A and B). Average number of minutes spent running per day was not significantly different between HR and C females, but HR males ran for more minutes than C males (Fig. 7C and D). Average speed of wheel running was higher in HR females than C females and higher in HR males than C males (Fig. 7E and F). No aspect of adult wheel running (total distance, duration, average running speed, maximum speed in any one-min interval [results not shown]) was statistically affected by maternal WD, with no interaction between linetype and WD. Analysis of wheel running by replicate lines are presented in Section 3.11.
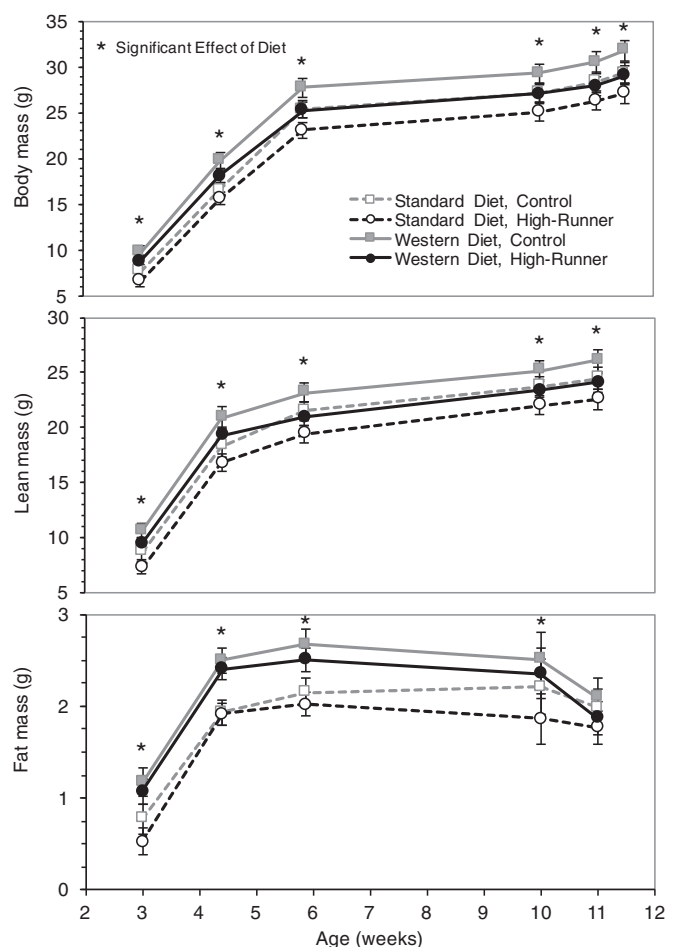

Fig. 4. Focal male body composition (total body mass, lean mass, and fat mass), adjusted for age. 3 weeks of age corresponds to weaning and 10 weeks of age represents measurements when mice were first granted adult wheel access (though this ranged from 9 to 10 weeks old), and 11 weeks represents measurements after 6 days of wheel access. Age at approximately 11.5 weeks represents measurements at sacrifice (top panel only). Each point represents a LSM for ~25 males and error bars are standard errors. Open points and dashed lines indicate maternal standard diet, solid points and lines indicate maternal Western diet, control mice are in grey, and high runner mice are in black. 2-tailed $P < 0.05$ for effect of maternal diet at the time points indicated by an asterisk (*).

3.9. Organ masses

Adjusting for body mass and age, heart ventricle mass was increased by maternal WD, with no effect of linetype and no interaction (Table 1). Posterior subcutaneous fat pad, abdominal pelvic fat pad, liver, spleen, and brain masses were unaffected by maternal WD (Table 1). As reported previously [56], HR mice tended to have larger brains than C mice. Mice with the mini-muscle phenotype had larger hearts, livers, spleens, and both fat pads (Table 1), with several of these effects being reported previously (see Discussion).

3.10. Plasma hormone concentrations

With age and days from the end of wheel access to sacrifice as covariates, plasma leptin, adiponectin, and corticosterone concentrations were unaffected by maternal WD and did not differ between HR and C lines, with no interaction (Online Supplemental Table 2).

3.11. Line analyses of wheel running

Analyses of variation among the replicate lines were performed separately by sex and linetype (Fig. 8). These analyses revealed that
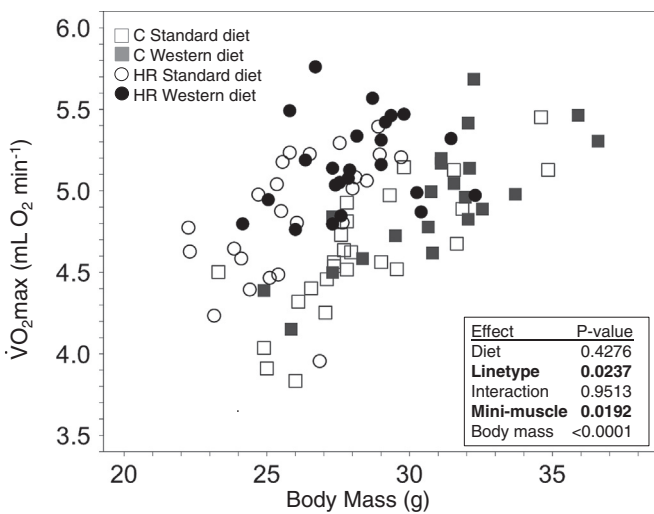
Fig. 5. Maximal aerobic capacity (VO₂max) measured at ~8 weeks of age for focal males. N = 91, approximately equal number in each group. Open points indicate maternal standard diet, solid points indicate maternal Western diet, control mice are in grey, and high runner (HR) mice are in black. Results of statistical analyses (fixed effects) are shown on the right. All analyses included covariates of age (not shown).

replicate lines of C (N = 4) and HR (N = 4) mice responded differently to maternal WD, and that these responses were influenced by sex.

For wheel running (mean revolutions/day on days 5 & 6) by C females, maternal WD had no statistical effect and replicate lines did not differ significantly (Fig. 8). In HR females, however, maternal WD significantly increased wheel running in one line (HR line 7). In C males, maternal WD increased wheel running, and lines differed significantly. In HR males, maternal WD did not significantly affect wheel running, but lines differed significantly.

We analyzed the number of intervals run per day (1-min intervals containing at least one revolution), the mean wheel-running speed (revolutions/intervals), and the maximum speed (single highest 1-min interval), as averages of days 5 & 6 (Online Supplemental Figs. 1–3). We also analyzed body mass by replicate line (Online Supplemental Fig. 4).

3.12. Effects on grand-offspring

To measure the effects of grand-maternal diet, males and females whose dams had the same diet (SD or WD) were paired to breed. Grand-offspring body mass and wheel running as adults were unaffected by grand-maternal WD when analyzed by linetype (Online Supplemental Table 3).

Line analyses of grand-offspring wheel running revealed that replicate lines of HR mice were affected by grand-maternal WD, but only in female mice (Fig. 9). Specifically, maternal WD significantly increased wheel running for females in HR line 7 and significantly decreased it for females in HR line 3 (differences of least squares means from SAS Procedure Mixed). C females were not affected by grand-maternal WD and replicate lines did not differ significantly. In C and HR males, maternal WD did not affect wheel running but replicate lines differed significantly. We also analyzed grand-offspring body mass by sex and replicate line, but found no differences due to grand-maternal diet (Online Supplemental Fig. 5).

4. Discussion

We tested the general hypothesis that early-life exposure to maternal Western diet (beginning prior to conception and lasting through ~2/3 of the lactation period) would affect adult offspring body composition, levels of physical activity, and associated subordinate traits (e.g., organ masses and hormone levels). We expected that any such effects would be mediated by genetic background; in particular, we predicted that the four selectively bred high runner lines of mice would be somewhat protected against negative consequences of maternal overnutrition as compared with the four non-selected Control lines. For most measures, we obtained data on ~100 focal adult males, but for adult wheel running and body mass we also obtained data for ~200 additional males and ~300 total females.

4.1. Maternal diet changes offspring body composition

Maternal WD had long-lasting effects on the morphology of offspring, increasing fat mass, lean mass, and percent fat of body mass well into adulthood (Fig. 4). Fat % has been found to be increased by maternal high-fat diets in rats [7,10], but to our knowledge, our study is the first to report increased lean mass. As illustrated in Fig. 4 and in Online Supplemental Table 1, maternal WD significantly increased absolute fat mass at weaning, at 4.5 weeks of age, at 5.5 weeks of age, and at ~10 weeks of age (all P < 0.01), which was immediately prior to the start of wheel access. However, the effect on fat mass was no longer statistically significant after wheel access (P = 0.28). Moreover, the absolute amount of fat lost during wheel access was significantly greater for mice whose dams had experienced WD (Online Supplemental Table 1). Thus, even acute exercise can reverse the negative effect of maternal WD on fat mass, and this occurred for both HR and C mice. In future studies, it would be of considerable interest to determine
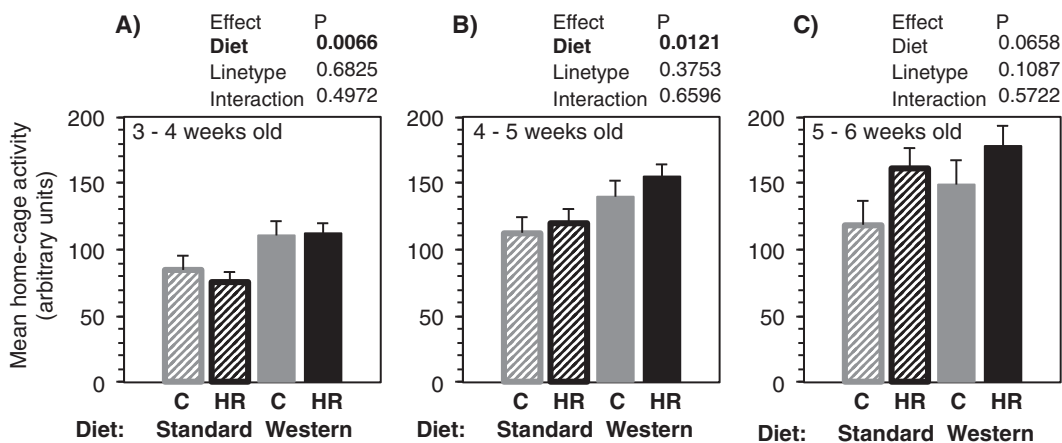

Fig. 6. Juvenile home-cage activity (measure of spontaneous physical activity, in arbitrary units, averaged across days in the week) for focal males. Panels show 3 consecutive weeks. Bars are age-adjusted LSM + SE, N = 25 in each group. Striped bars indicate maternal standard diet, solid bars indicate maternal Western diet, grey bars are control mice, and black bars are high runner (HR) mice. Results of statistical analyses (fixed effects) are shown above each graph. All analyses included age as a covariate (P values not shown).
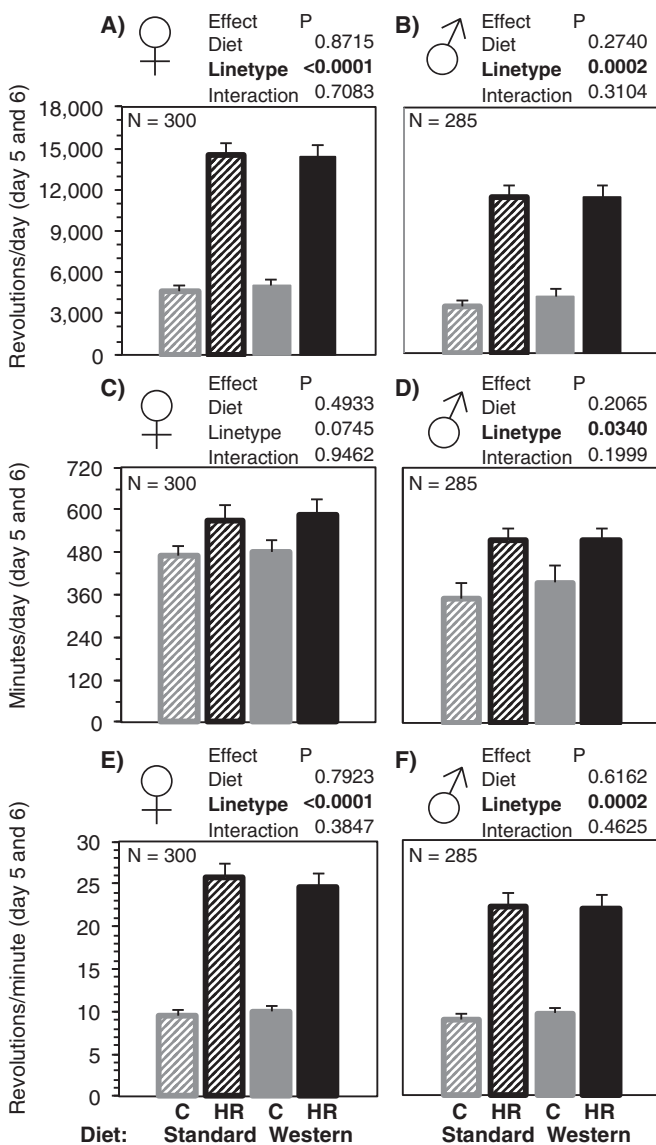
Fig. 7. Total voluntary wheel running revolutions, duration (number of 1-min intervals with at least one revolution), and average speed of days 5 + 6 of 6 days of wheel access of adult C and HR mice given maternal SD or WD, with separate analyses for females and males. Bars are age- and wheel-freeness-adjusted LSM + SE and N were approximately equal for each group. Striped bars indicate maternal standard diet, solid bars indicate maternal Western diet, grey bars are control mice, and black bars are high runner (HR) mice. Results of statistical analyses (fixed effects) are shown above each graph. All analyses included age as a covariate (P values not shown).

if the beneficial effect of acute adult exercise persists.

Maternal WD increased adult heart ventricle mass, even after adjusting for variation in body mass by ANCOVA. Although we have not tested whether this increase was beneficial (physiological) or detrimental (pathological), other studies of mice have reported that maternal high-fat, high-sugar diet caused pathological increases in ventricle mass [57,58]. In any case, maternal WD did not increase offspring maximal aerobic capacity, which should generally correlate positively with heart size (e.g., [37]). Interestingly, we found that individuals with the mini-muscle phenotype (found only in two of the HR lines in the present sample of mice) had statistically higher VO2max. Previous studies have generally not found this to be the case [43,53], although mini-muscle individuals did have higher VO2max when tested in hypoxia [37] and in a comparison of only one control and two HR lines [54].

4.2. Maternal diet apparently changes offspring energy balance

Maternal WD increased juvenile (3–6 week old) home-cage activity. All mice became more active with age, and at 5–6 weeks HR tended to be more active than C, as expected from previous studies when these mice are housed without wheels [34,45,46,59]. However, consumption of standard chow, measured only during the juvenile stage for focal males and analyzed using body mass as a covariate, was not affected by maternal WD and did not differ between HR and C mice (Online Supplemental Table 1). This result is consistent with a previous study of mice which reported that maternal over-nutrition increased offspring preference for high-fat food but did not change intake of control food [60]. Given that offspring spontaneous physical activity was increased by maternal WD, coincident with increased offspring body mass (Fig. 4, weeks 3–6) but no change in juvenile food consumption (adjusted for variation in body mass), it is possible that basal or resting metabolic rate was reduced by maternal WD.

In general, maternal Western diet did not affect voluntary exercise on wheels by adult offspring. However, we did find line- and sex-specific effects. Specifically, maternal WD increased wheel running for female offspring of HR line 7, which emphasizes our previous findings that the replicate HR lines and the two sexes have undergone somewhat different evolutionary paths under the same selection regime [49–51]. A sex-specific effect was also found in a study of rats with maternal high-fat diet, which reported less active male and more active female offspring when given free wheel access for one week [8]. Furthermore, grand-offspring of the dams fed WD had significantly altered wheel running in two HR lines (Fig. 9). Maternal WD significantly increased wheel running in HR line 7 offspring and grand-offspring. In HR line 3, maternal WD only tended to decrease running in the offspring, but significantly decreased running in the grand-offspring, showing an "amplifying" effect through two generations. (Interestingly, line 3 is fixed for the mini-muscle allele [52].) Transgenerational amplification

Table 1

Significance levels (P values) from statistical analyses of adult internal organ masses of mice after 6 days of wheel access. Log10 body mass, age, and days from the end of wheel access to sacrifice were used as covariates (results not shown). The grand mean of body mass was 29.90 g with standard deviation = 3.16 g (N = 92). Groups were approximately equal HR and C, with maternal SD or WD. Signs following P values indicate direction of effect: for diet, "+" indicates Western diet > standard diet; for linetype, "+" indicates high runner lines > control lines. The bold-face indicates a P-value of less than 0.05.

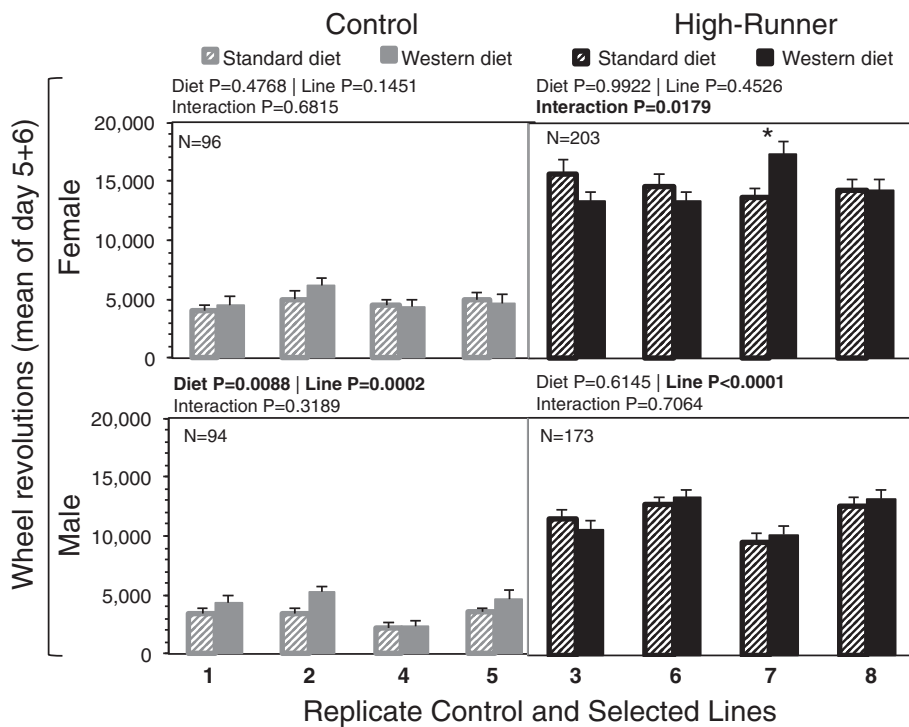| Organ mass (log10 transformed)	N	P values of effects				
		Body mass	Diet	Linetype	Interaction	Mini
Abdominal pelvic fat pad	92	< **0.0001** +	0.8054 −	0.7513 −	0.4243	**0.0380** +
Posterior subcutaneous fat pad	91	< **0.0001** +	0.6195 +	0.9624 −	0.4475	**0.0371** +
Heart ventricle	92	< **0.0001** +	**0.0050** +	0.2327 +	0.4932	**0.0005** +
Liver	92	< **0.0001** +	0.1638 −	0.9518 −	0.9902	**0.0070** +
Spleen	89	< **0.0001** +	0.0578 −	0.6383 −	0.0932	**0.0014** +
Total brain	91	< **0.0001** +	0.3560 +	0.0680 +	0.3159	0.1455 +
Cerebellum	91	**0.0101** +	0.6049 −	0.2192 +	0.2955	0.9785 +
Non-cerebellar brain	91	**0.0001** +	0.2636 +	0.1516 +	0.7370	0.0825 +

Fig. 8. Total wheel revolutions of adult mice given maternal standard diet (striped bars) or Western diet (solid bars), with separate analyses for sex and replicate control (in grey) and high runner (in black) lines. Bars are LSM + SE (age and wheel freeness used as covariates). Total N is shown in the upper left-hand corner, and Ns were approximately equal for each line within linetype and diet within line. Results of statistical analyses (fixed effects) are shown above each graph. Asterisks indicate differences of LSM within each line between diets ($P < 0.05$) from SAS Procedure Mixed.
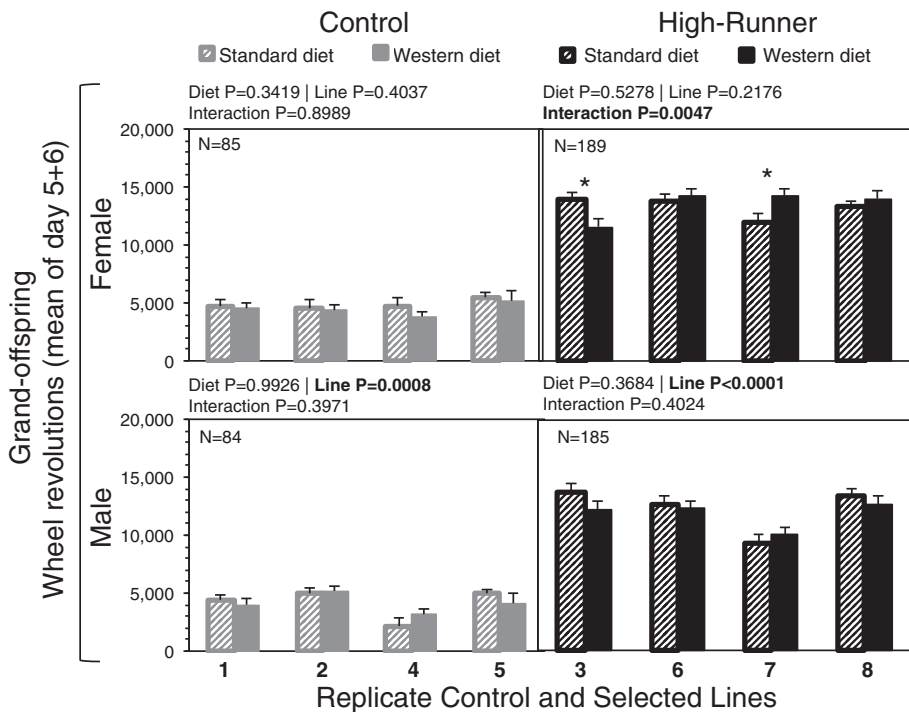

Fig. 9. Total wheel revolutions of grand-offspring of dams given standard diet (striped bars) or Western diet (solid bars), with separate analyses for sex and replicate control (in grey) and high runner (in black) lines. Grand-offspring were produced by mating males and females within each line whose dams had the same diet. Bars are LSM + SE (age and wheel freeness used as covariates). Total N is shown in the upper left-hand corner, and Ns were approximately equal for each subgroup. Results of statistical analyses (fixed effects) are shown above each graph. Asterisks indicate differences of LSM within each line between diets ($P < 0.05$) from SAS Procedure Mixed.

of obesity in mice can be mediated by epigenetic alterations via DNA methylation [61], which could be the case for activity levels as well.

Two previous studies on these lines of mice have reported that cage activity is reduced when rodents are housed with wheels [45,46]. In the present study, we only measured home-cage activity of adults with wheel access and found no statistical effect of maternal WD and no difference between HR and C mice (Online Supplemental Table 1), the latter result consistent with previous reports [45,46]. Another study of mice, housed without wheels, reported that a maternal high-fat, high-sugar diet decreased spontaneous physical activity of adult offspring measured over one week via telemetry [24].

4.3. No apparent effects of maternal diet on offspring hormone levels

Hormone concentrations of offspring were measured 6–16 days after 6 days of adult wheel access, which may have influenced results. As stated in Section 4.1, after 6 days of wheel access, fat mass no longer differed between groups. Additionally, we found no effect of maternal WD on fat pad masses measured at dissection (Table 1). In general, body fat is a positive predictor of leptin levels in mice, including in previous studies of the lines we studied [45,62]. As expected, we found that leptin levels were positively associated with the masses of both posterior subcutaneous fat pads and abdominal pelvic fat pads (Online

Supplemental Table 2). Therefore, leptin levels may have been affected by maternal WD before adult wheel access, but been returned to "baseline" values due to exercise-related fat loss. Alternatively, the lack of effect of maternal WD might be a common outcome, as a review of rat studies with maternal high-fat diet reported that 4 of 8 studies did not find significant effects on plasma leptin levels [63].

One previous study of ICR mice (the base population for our selection experiment) found that maternal WD decreased circulating adiponectin concentrations in adult offspring [15], but we did not observe such an effect. Circulating adiponectin concentration is expected to be strongly negatively related with body fat (e.g., [64,65]), which, as noted in the previous paragraph, was not affected by maternal WD after 6 days of adult wheel access. Furthermore, we did not replicate the finding of a previous study that showed higher plasma adiponectin in HR versus C males [66].

Maternal high-fat diet has been reported to lower offspring circulating corticosterone levels in mice [13,14], but we did not observe this effect. Previous studies have reported that HR mice have higher circulating corticosterone levels than C mice (e.g., [34,67]), but we did not obtain this result (Online Supplemental Table 2), possibly because mice were measured after 6 days of wheel access, followed by several days of sedentary housing.

Physical activity is frequently a confounding factor in measurements of circulating hormone levels. Ideally, we would have taken blood samples prior to adult wheel testing, but we chose not take any blood samples at that time because we did not want to risk affecting the wheel-running phenotype that was a crucial outcome variable for this study. Therefore, we cannot definitively conclude the lack of endocrine effects due to maternal WD. (Similar cautions would apply to organ masses, although we did find an effect of maternal WD on heart size.) Future studies should examine hormone levels at different stages of development for offspring of mothers with high-fat, high-sugar diets while including treatments of sedentary vs. active. Another approach would be to examine the density of central leptin receptors in the hypothalamus, given the differences in body fat mass resulting from maternal diet (Fig. 4, Online Supplemental Table 1).

4.4. Concluding remarks

In summary, we found that maternal Western diet can have long-lasting effects on offspring adult morphology and behavior, although these effects can be mediated by sex and genetic background. However, some effects, such as that observed for body fat (Fig. 4), can be reversed by as little as 6 days of wheel access. Use of the polygenic high runner mouse lines and their control counterparts allows for a wide scope of exploration of variation in the complex phenotypes related to activity levels, including the importance of population differences and effects of genetic background in interacting with environmental factors. To our knowledge, ours is the first study to characterize effects of maternal WD on mouse strains with inherent propensity for high levels of physical activity.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.

doi.org/10.1016/j.physbeh.2017.06.008.

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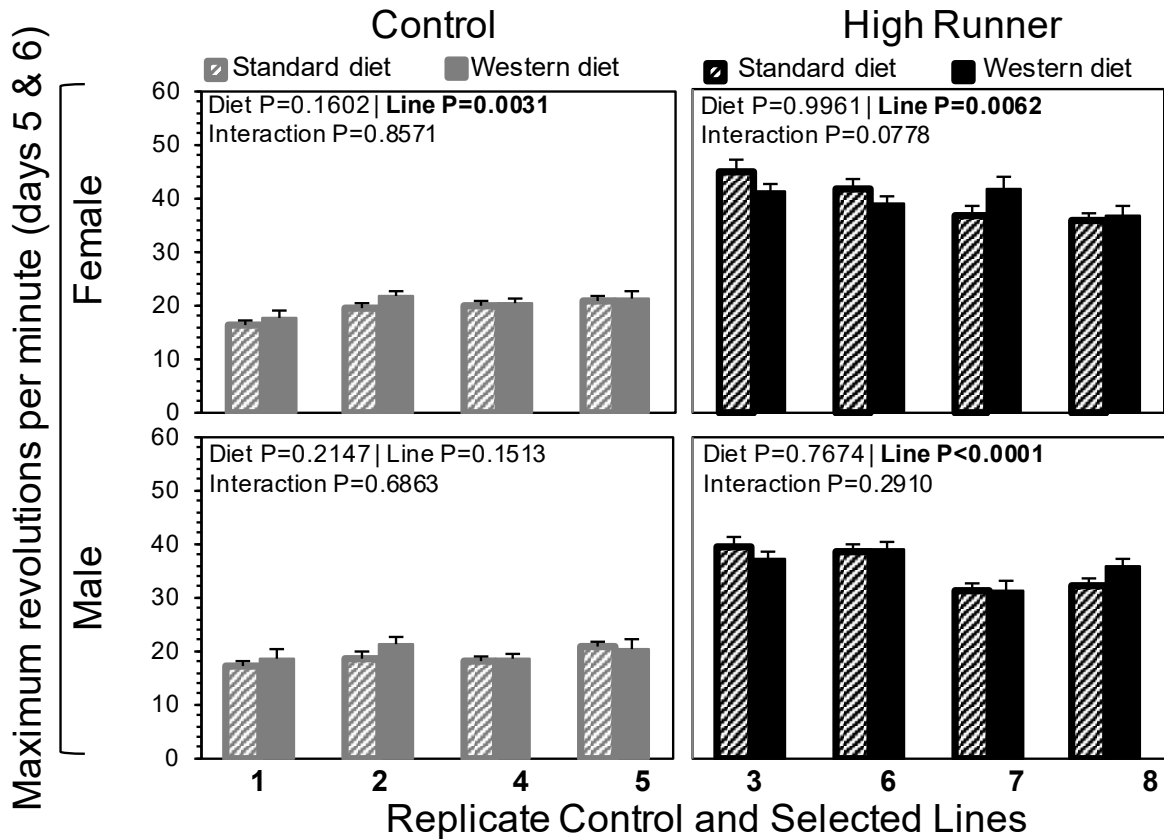
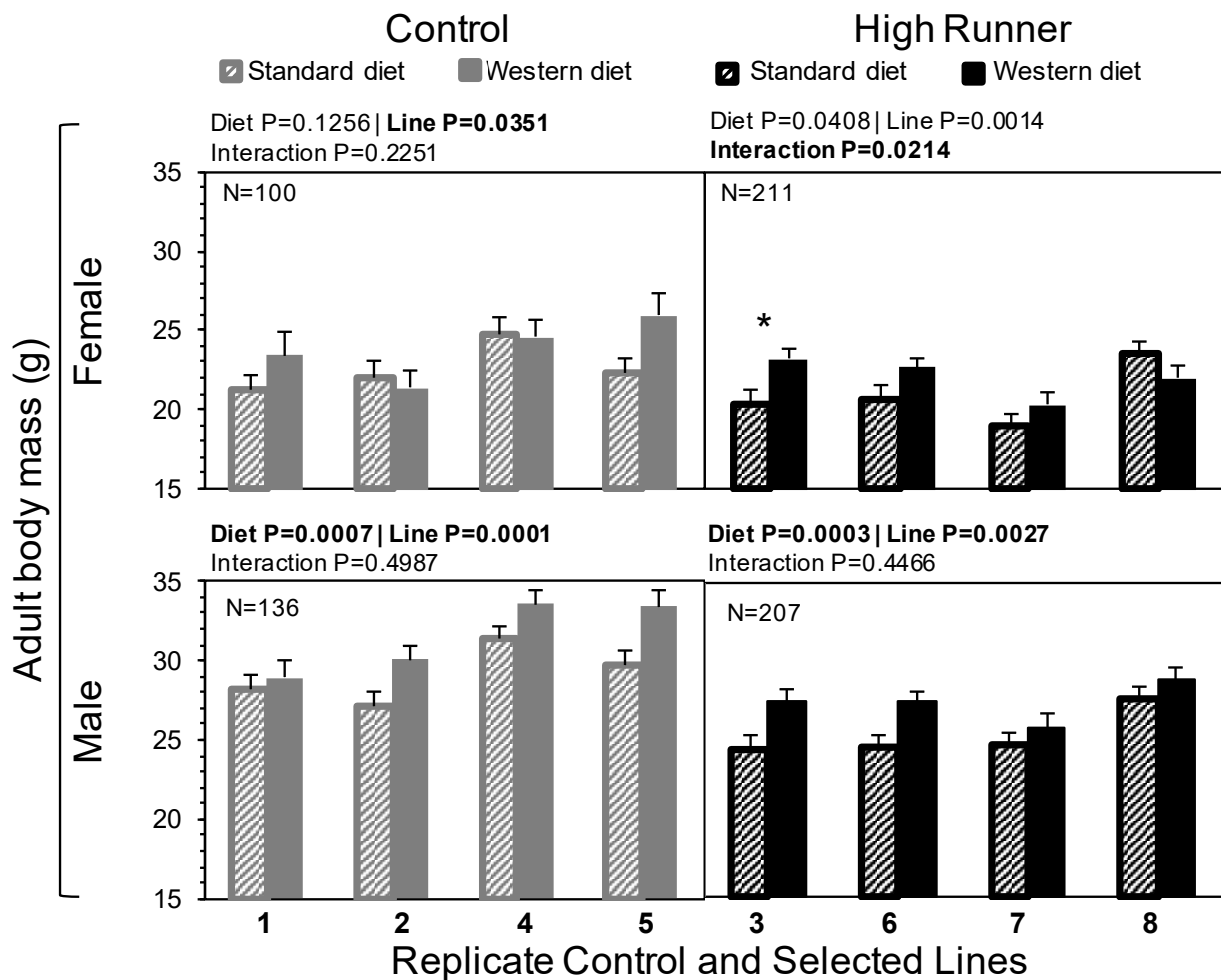## ONLINE SUPPLEMENTAL FIGURES AND TABLE
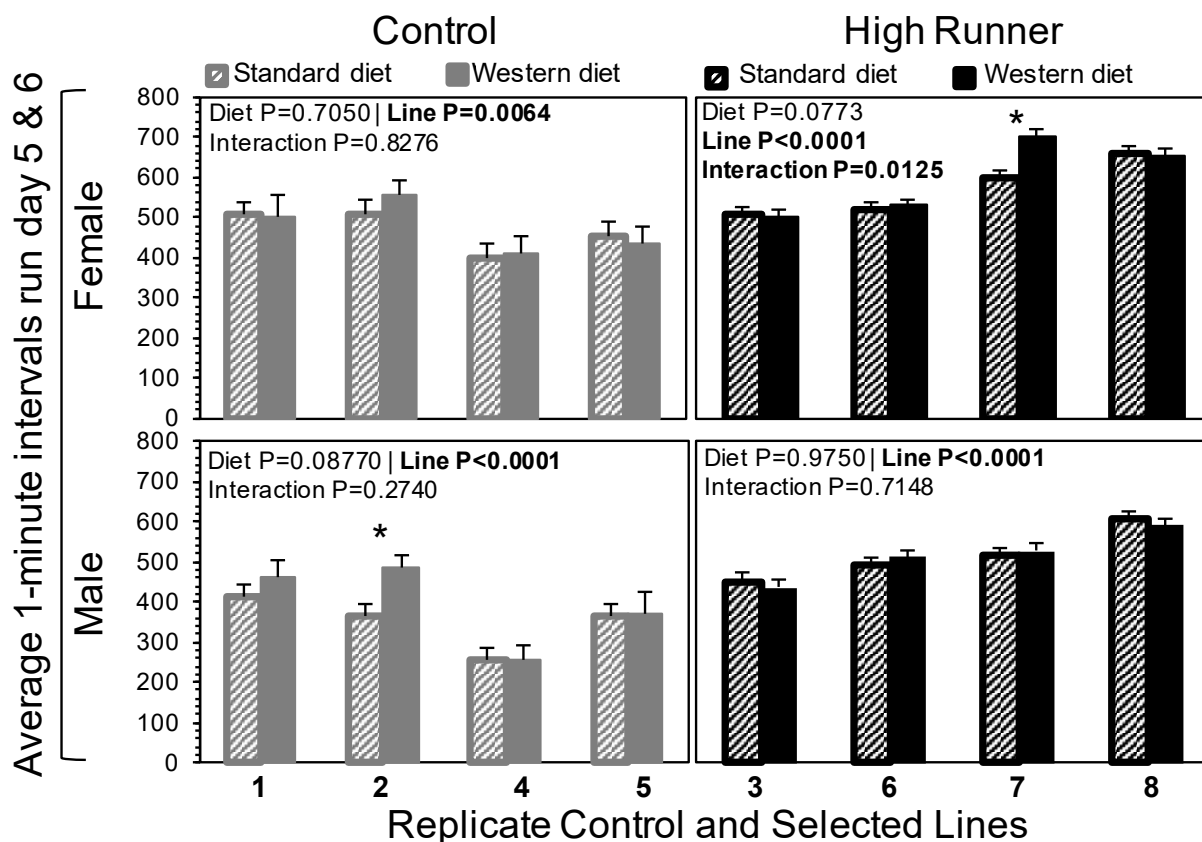
Online Supplemental Figure 1. Wheel running intervals per day, line analyses. Maternal WD did not overall affect C and HR females and males, but lines were significantly different in each group ($P < 0.007$), and HR females had an interaction between diet and line, with maternal WD significantly increasing intervals in HR line 7 ($P < 0.05$). LSM + SE adjusted for age and wheel freeness. Asterisks indicate differences of LSM within each line between diets ($P < 0.05$) from SAS Procedure Mixed. All analyses included covariates of age (not shown).
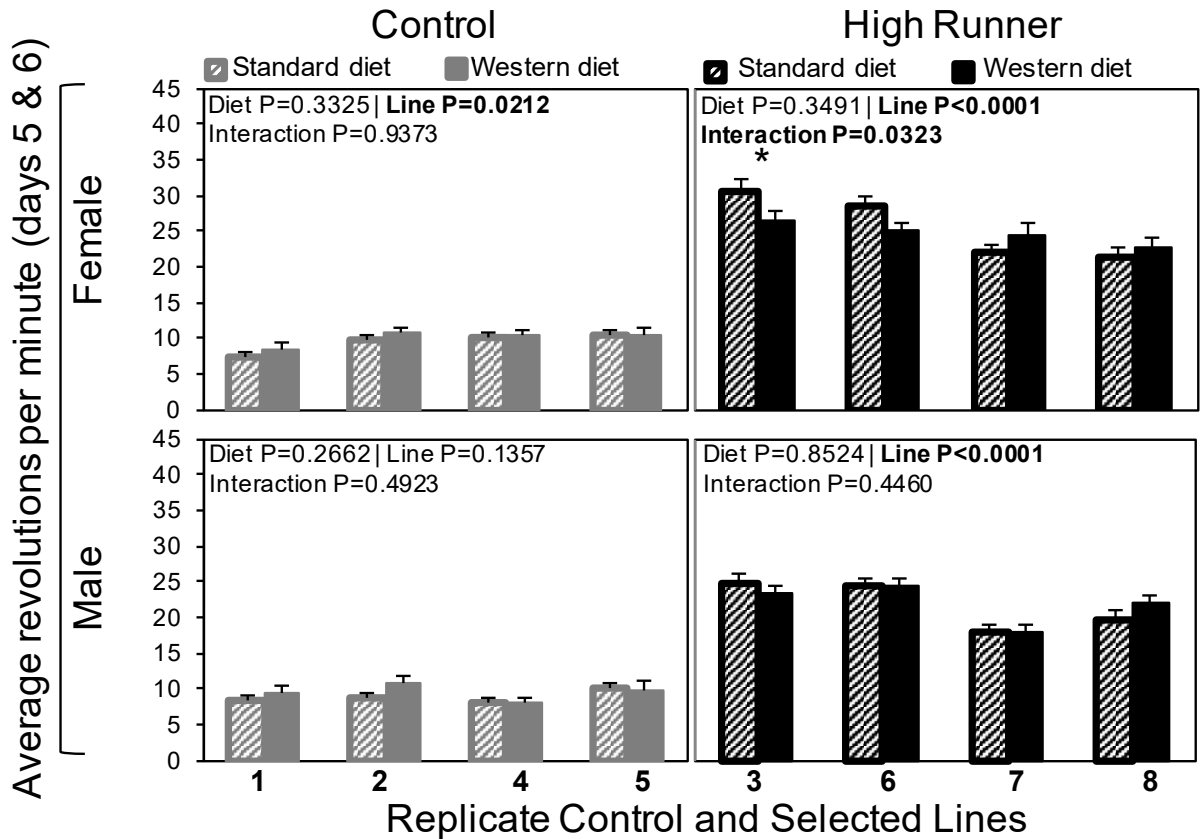
Online Supplemental Figure 2. Average wheel running speed (revolutions per minute), line analyses. Average speed in C males was unaffected by diet or line but C female, HR female, and HR male lines differed significantly within each group ($P < 0.05$). HR females had a significant interaction between diet and line ($P = 0.0323$), with maternal WD significantly decreasing average speed in HR line 3 ($P < 0.05$). LSM + SE adjusted for age and wheel freeness. Asterisks indicate differences of LSM within each line between diets ($P < 0.05$) from SAS Procedure Mixed. All analyses included covariates of age (not shown).

Online Supplemental Figure 3. Maximum wheel running speed (revolutions per minute), line analyses. Maximum speed in C males was unaffected by diet or line, but C female, HR female, and HR male lines differed significantly within each group ($P < 0.05$). LSM + SE adjusted for age and wheel freeness. All analyses included covariates of age (not shown).

Online Supplemental Fig. 4. Body mass of adult mice given maternal standard diet (striped bars) or Western diet (solid bars), with separate analyses for sex and replicate control (in grey) and high runner (in black) lines. Bars are LSM + SE (age used as covariate). Total N is shown in the upper left-hand corner, and Ns were approximately equal for each group. Results of statistical analyses (fixed effects) are shown above each graph. Asterisks indicate differences of LSM within each line between diets (*P* < 0.05) from SAS Procedure Mixed. All analyses included covariates of age (not shown).

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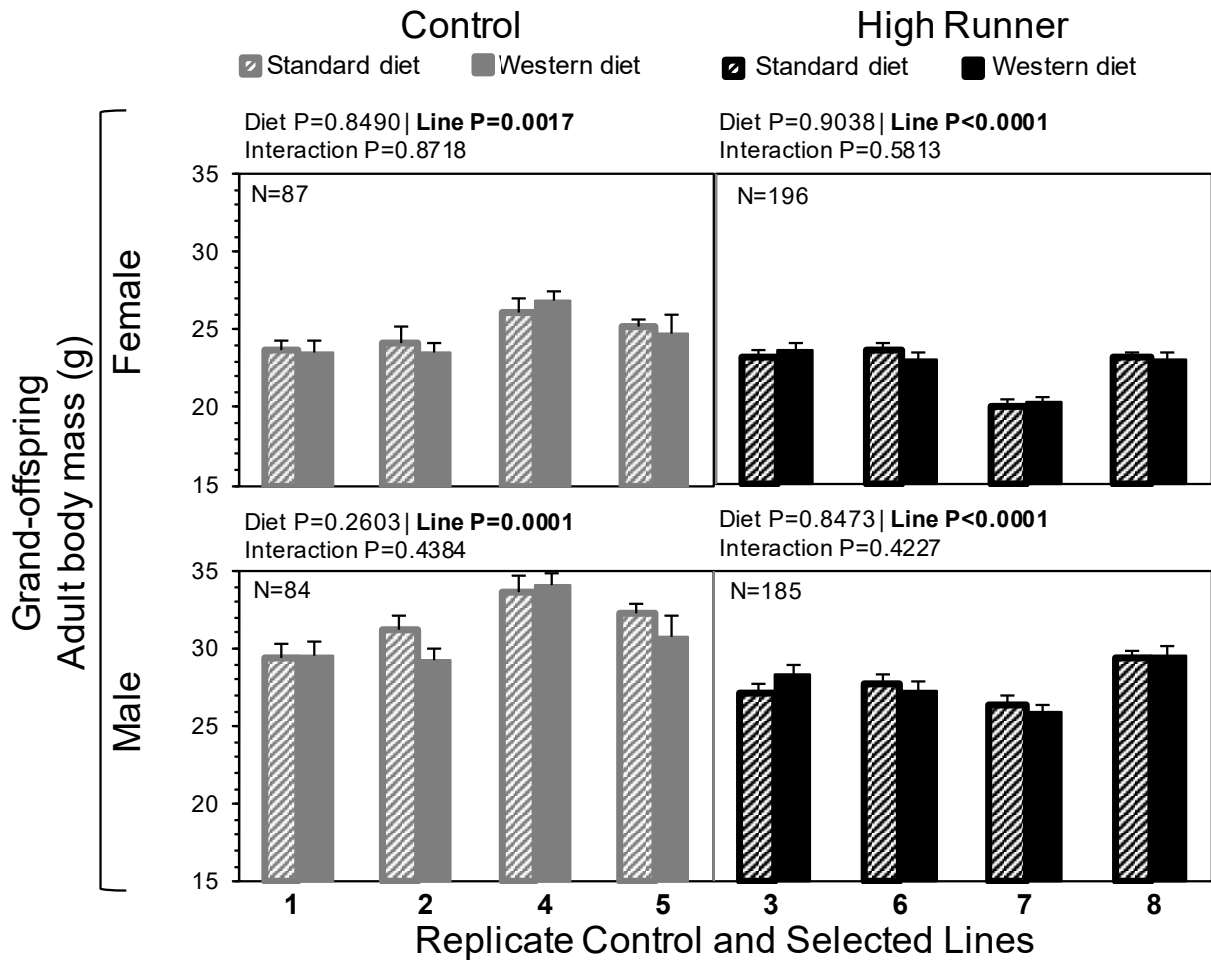
Online Supplemental Fig. 5. Adult body mass of grand-offspring of dams given standard diet (striped bars) or Western diet (solid bars), with separate analyses for sex and replicate control (in grey) and high runner (in black) lines. Grand-offspring were produced by mating males and females within each line whose dams had the same diet. Bars are LSM + SE (age used as a covariate). Total N is shown in the upper left-hand corner, and Ns were approximately equal for each group. Results of statistical analyses (fixed effects) are shown above each graph. Asterisks indicate differences of LSM within each line between diets ($P < 0.05$) from SAS Procedure Mixed. All analyses included covariates of age (not shown).
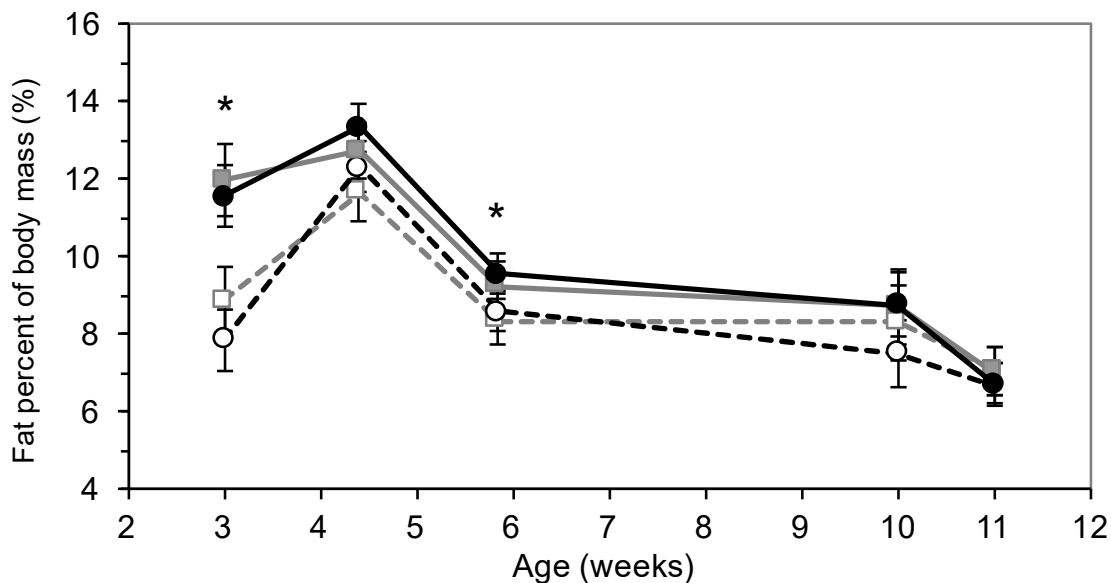
Online Supplemental Fig. 6. Focal male body fat percentage adjusted for age (total body mass, lean mass, and fat mass data are in Fig. 4 in the manuscript). 3 weeks of age corresponds to weaning and 10 weeks of age represents measurements when mice were first granted adult wheel access (though this ranged from 9-10 weeks old), and 11 weeks represents measurements after 6 days of wheel access. Age at approximately 11.5 weeks represents measurements at sacrifice. Each point represents a LSM for ~25 males and error bars are standard errors. Open points and dashed lines indicate maternal standard diet, filled in points and solid bars indicate maternal Western diet, control mice are in grey, and high runner mice are in black. 2-tailed $P < 0.05$ for effect of maternal diet at the time points indicated by an asterisk (*).

Online Supplemental Table 1. Significance levels (*P* values) from statistical analyses of various physiological and behavioral tests on mice whose dams were given Western. Main effects were maternal diet (WD or SD), linetype (HR or C), and their interaction. Signs indicate direction of effect (indicated only for *P* < 0.1) and all statistically significant *P* values (<0 .05) are in bold. All analyses included age as a random variable, and additional covariates were used where appropriate, including body mass, mini-muscle status, litter size, and sensitivity of home-cage sensors. All analyses included covariates of age (not shown).

Trait	Sex	N	Diet	Linetype	Interaction	Body mass	Mini	Other
Litter characteristics								
First pup move on own	Both	97	0.9411	0.9547	0.9096			litter size 0.0713-
First pup feed on own	Both	95	**0.0010+**	**0.0155-**	**0.0054**			litter size 0.0983+
First pup with eyes open	Both	93	0.0588+	0.3041	0.9398			litter size 0.2845
Dam mass at weaning	Dams	95	0.2052	0.6342	0.6527			litter size **0.0026+**
Pair-to-birth interval	Dams	100	0.9639	0.0941+	0.2730			
Pup sex ratio	Dams	119	0.1274	0.7159	0.4033			
Focal male body composition								
Body mass at weaning	Male	97	**0.0134+**	0.1629	0.9492		0.9817	
4.5 weeks	Male	96	**0.0018+**	0.1383	0.5199		0.7189	
5.5 weeks	Male	97	**0.0021+**	0.0828-	0.7811		0.2379	
Start of wheel access	Male	95	**0.0017+**	0.1403	0.8796		0.0704-	
After wheel access	Male	94	**0.0026+**	0.1592	0.5201		0.2391	
Sacrifice	Male	93	**0.0027+**	0.1386	0.5675		0.1219	
Lean mass at weaning	Male	95	**0.0088+**	0.0738-	0.8130		0.7121	
4.5 weeks	Male	96	**0.0021+**	0.1856	0.9002		0.5158	
5.5 weeks	Male	95	**0.0027+**	0.0822-	0.8247		0.0780-	
Start of wheel access	Male	94	**0.0059+**	0.1589	0.8463		**0.0221-**	
After wheel access	Male	93	**0.0031+**	0.1574	0.9127		**0.0472-**	
Fat mass at weaning	Male	97	**0.0097+**	0.3084	0.5841		0.7222	
4.5 weeks	Male	93	**0.0003+**	0.7204	0.6346		0.0560+	
5.5 weeks	Male	96	**0.0023+**	0.3663	0.8681		0.1207	
Start of wheel access	Male	94	**0.0098+**	0.5263	0.3842		0.8503	
After wheel access	Male	93	0.2839	0.3912	0.9503		**0.0018+**	
Fat percent at weaning	Male	91	**0.0003+**	0.5160	0.5333		0.4877	
4.5 weeks	Male	94	0.0889+	0.4018	0.9746		**0.0155+**	
5.5 weeks	Male	94	**0.0168+**	0.6965	0.9106		0.0655+	
Start of wheel access	Male	94	0.0566+	0.7649	0.2822		0.4864	
After wheel access	Male	93	0.9614	0.6383	0.9524		**<0.0001+**	
Change in body composition before vs. after 6 days of wheel access								
Total mass (absolute)	Female	308	0.7503	0.6913	0.7943			
Total mass (% change)	Female	305	0.8806	0.8680	0.7911			
Total mass (absolute)	Male	341	0.1537	0.7070	0.8513			
Total mass (% change)	Male	341	0.1308	0.7254	0.8372			

	Sex	N						Sensor sensitivity
Lean mass (absolute)	Male	93	0.3164	0.4462	0.9463		0.2976	
Lean mass (% change)	Male	93	0.4140	0.4625	0.9470		0.1654	
Fat mass (absolute)	Male	93	**0.0340+$**	0.3683	0.3728		**0.0297-**	
Fat mass (% change)	Male	93	0.0911+	0.6388	0.1649		**0.0047-**	
Fat % (absolute)	Male	93	0.0636+	0.4480	0.3038		0.1019	
Fat % (% change)	Male	93	0.0938+	0.5944	0.1835		**0.0091-**	
Juvenile food consumption								
3-4.5wks	Male	92	0.5656	0.1443	0.3487	**<0.0001**	0.6453	
4.5-6wks	Male	91	0.4173	0.2233	0.5197	**<0.0001**	0.1410	
Elevated plus maze								
Total distance	Male	94	0.4745	0.2298	0.4452		0.6977	
% time in center	Male	94	0.8482	0.6350	0.7149		0.1451	
% time in open arms	Male	94	0.5962	0.8614	0.2095		0.4891	
% entries into open arms	Male	93	0.5828	0.6667	0.5474		0.4763	
% distance in open arms	Male	93	0.5752	0.9566	0.2927		0.7151	
Velocity in open arms	Male	92	0.2388	0.6103	0.7097		0.4973	
Velocity in closed arms	Male	93	0.3473	0.2542	0.4156		0.7114	
Fecal pellets	Male	93	0.7894	0.0784	0.9984		0.8283	
Fecal pellets + urine pools	Male	93	0.8721	0.3054	0.7111		0.9174	
Adult home-cage activity	Male	89	0.5770	0.8249	0.2431		0.1219	0.5178
with wheel revolutions&	Male	65	0.5474	0.5538	0.1175		0.1325	0.2757

$ All mice lost fat, and mice with maternal WD lost more fat.

&Wheel revolutions per day was an additional covariate (P = 03795), missing for a quarter of the mice.

Online Supplemental Table 2. Significance levels (*P* values) from statistical analyses of plasma concentrations of three hormones: leptin, adiponectin, and corticosterone. All analyses used age and number of days from the end of wheel access to sacrifice as covariates (results not shown). Bleed delay time was less than 2 minutes, and did not significantly affect corticosterone concentrations (results not shown). Sample size was N = 91-93, approximately equally distributed among experimental groups. Sign after significant P values indicates direction of effect.

Hormone	*P* values of effects				
	Diet	Linetype	Interaction	Mini	Other
Leptin (square root ng/ml)	0.2811	0.3424	0.4349	0.1012	
with body mass	0.7169	0.8420	0.2817	**0.0150+**	**Mass P<0.0001+**
with posterior subcutaneous (PS) fat pad	0.5476	0.6213	0.2284	0.2548	**PS P<0.0001+**
with abdominal pelvic (AP) fat pad	0.9944	0.9385	0.4316	0.1530	**AP P<0.0001+**
with PS and AP fat pads	0.9899	0.8781	0.2367	0.2558	**PS P=0.0002+**
					AP P=0.0023+
Adiponectin (square root mg/ml)	0.6008	0.3579	0.3873	0.1829	
with body mass	0.8277	0.5793	0.4354	0.2620	Mass P=0.1361
with posterior subcutaneous (PS) fat pad	0.7617	0.3486	0.3335	0.1982	PS P<0.7426
with abdominal pelvic (AP) fat pad	0.5159	0.3146	0.3951	0.2042	AP P<0.5999
with PS and AP fat pads	0.6288	0.3107	0.3602	0.1888	PS P=0.4643
					AP P=0.3296
Corticosterone ($\log_{10}$ ng/ml)	0.7406	0.1504	0.2525	0.3257	

Online Supplemental Table 3. Significance levels (*P* values) from statistical analyses of grand-offspring body mass and wheel running as adults. Grand-offspring were produced by mating males and females within each line whose dams had the same diet. All analyses used age as a covariate and wheel-running analyses also used wheel freeness. Groups were approximately equally HR and C, with maternal SD and WD.

Trait	N	Diet	Linetype	Interaction	Age	Wheel Freeness
				P values of effects		
Female grand-offspring						
Wheel running (day 5&6)	276	0.8083-	**<0.0001+**	0.3742	0.1758-	**0.0015+**
Adult body mass	283	0.9777-	0.0772-	0.8924	**<0.0001+**	
Adult body mass after wheel access	282	0.3610-	0.2118-	0.9095	**0.0027+**	
Male grand-offspring						
Wheel running (day 5&6)	267	0.6984-	**0.0002+**	0.8705	**0.0079-**	0.6541+
Adult body mass	331	0.7675-	**0.0460-**	0.2688	**<0.0001+**	
Adult body mass after wheel access	270	0.3022-	**0.0242-**	0.3968	**<0.0001+**	