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Effects of early-life exposure to Western diet and voluntary exercise on adult activity levels, exercise physiology, and associated traits in selectively bred High Runner mice

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ABSTRACT

Exercise behavior is under partial genetic control, but it is also affected by numerous environmental factors, potentially including early-life experiences whose effects persist into adulthood. We studied genetic and early-life environmental effects on wheel-running behavior in a mouse model that includes four replicate high runner (HR) lines selectively bred for increased voluntary wheel running as young adults and four non-selected control (C) lines. In a full factorial design, mice from each line were granted wheel access or not and administered either standard or Western diet (WD) from weaning (3 weeks old) to 6 weeks of age (sexual maturity). In addition to acute effects, after a washout period of 8 weeks (~6 human years) in which all mice had standard diet and no wheel access, we found both beneficial and detrimental effects of these early-life exposures. During the first week of treatments, WD increased distance run by 29% in C mice and 48% in HR mice (significant Diet × Linetype interaction), but diet effects disappeared by the third week. Across the three weeks of juvenile treatment, WD significantly increased fat mass (with lean mass as a covariate). Tested as adults, early-life exercise increased wheel running of C mice but not HR mice in the first week. Early-life exercise also reduced adult anxiety-like behavior and increased adult fasted blood glucose levels, triceps surae mass, subdermal fat pad mass, and brain mass, but decreased heart ventricle mass. Using fat mass as a covariate, early-life exercise treatment increased adult leptin concentration. In contrast, early-life WD increased adult wheel running of HR mice but not C mice. Early-life WD also increased adult lean mass and adult preference for Western diet in all groups. Surprisingly, early-life treatment had no significant effect on adult body fat or maximal aerobic capacity (VO_2max). No previous study has tested for combined or interactive effects of early-life WD and exercise. Our results demonstrate that both factors can have long-lasting effects on adult voluntary exercise and related phenotypes, and that these effects are modulated by genetic background. Overall, the long-lasting effects of early-life exercise were more pervasive than those of WD, suggesting critical opportunities for health intervention in childhood habits, as well as possible threats from modern challenges. These results may be relevant for understanding potential effects of activity reductions and dietary changes associated with the obesity epidemic and COVID-19 pandemic.

1. Introduction

Human obesity and most of its negative health consequences result from complex interactions among diet, physical activity, environmental exposures, sex, genetic predisposition, and sociocultural factors [1–16]. Of these, declining physical activity and "Western" diets (high in fat & simple sugars) are seen as key contributors to increasing rates of obesity and its comorbidities in all age groups across multiple populations [17–21]. Conversely, a "good" diet (e.g., relatively low in sugar and fat (but see Esposito et al. [22]; O'Neill and Raggi [23]) and higher activity

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levels promote physical fitness and cardiovascular health while lowering the risk for obesity, Type 2 diabetes, and their comorbidities [24–28]. Importantly, not only voluntary exercise (VE) but also incidental or spontaneous physical activity (SPA) has beneficial effects in both animal models and humans (e.g., [28,29–32,33,34]).

Like obesity itself, human physical activity and diet choices are products of both genes and numerous environmental effects acting throughout an individual's development [32,35]. Many key environmental factors are experienced early in life, including *in utero* [36]. Such ontogenetic factors can strongly contribute to the development of various unhealthy adult behaviors, habits, diseases, and disorders [37–48]. Unfortunately, interventions to improve diet or increase physical activity often have little impact on children's activity ([49,50]; but see [51,52]), and have not halted the increasing prevalence of obesity in children [53].

Human studies suggest that experiences occurring from conception to sexual maturity can alter adult levels of voluntary exercise and/or SPA. For example, adults who were exposed to the Dutch Famine *in utero* have increased adiposity and more atherogenic lipid profiles that may be related to decreased physical activity [15,54]. However, mechanisms by which early-life experiences affect physical activity are poorly understood [40,48,54–60], in part because existing human studies are mostly correlational, cross-sectional [35], or of questionable methodological quality (e.g., use of questionnaires to gage physical activity: [15,59,61]).

Numerous rodent studies establish that *in utero* and other early-life exposures to calorie-restricted, low-protein, high-fat, or high-carbohydrate diets can affect obesity-related measures in adults, such as body composition, appetite, dietary preferences, reward signaling, and the microbiome [46,62–68]. Far fewer studies address maternal or early-life effects on adult voluntary exercise or SPA ([65,69–71]; see also [72] on sheep).

Here, we aimed to identify possible long-lasting effects of juvenile exposure to Western diet (WD) and exercise. We used mice from a well-characterized experimental evolution [73] animal model: four replicate High Runner (HR) lines that were selectively bred for 76 generations for increased voluntary wheel-running behavior on days 5 and 6 of a 6-day wheel exposure as young adults and four non-selected control (C) lines [74,75]. On average, mice from the HR lines run ~3-fold greater distance per day compared with the C lines, for both sexes [75]. A number of other phenotypes have evolved in association with wheel-running behavior in the HR lines, including greater SPA when wheels are not provided, increased running endurance during forced treadmill exercise, increased maximal aerobic capacity during forced exercise (VO_2max), reduced body fat, larger hearts, altered dopamine signaling in the brain, altered endocannabinoid signaling, higher baseline circulating corticosterone levels, and lower circulating leptin levels [29,76–83].

Previously, we found that access to Western diet beginning at weaning and continuing through adulthood had a large stimulatory effect (~50–75%) on daily wheel-running distance of both juvenile and adult male HR mice, with no statistically significant effect on C mice ([84]; only males tested). These dramatic results for voluntary exercise were unprecedented for rodents, although in adult rats a high-fat diet can increase VO_2max and endurance capacity [85]. A subsequent study of HR and C mice found that early-life wheel access for just 3 weeks, starting at weaning, increased adult wheel running in males of both linetypes, with no effect on SPA and contrasting effects on adult circulating leptin levels in HR and C mice [86]. All early-life-exercise mice had significantly reduced adult body mass and a trend for reduced visceral fat pad mass [86]. Taken together, these previous results demonstrate that early-life effects on adult physical activity can be genotype-dependent.

In the present study, we addressed three specific questions. (1) Do WD and wheel access given to juveniles have acute/immediate effects on juvenile physical activity, caloric intake, and body composition? (2) Do WD and wheel access given to juveniles have long-lasting effects on adult physical activity and related phenotypes? (3) Do early-life diet or exercise interact with genetic backgrounds to influence adult phenotypes? From what has been reported in similar previous studies [84,86], we expected that WD and/or exercise would have substantial effects when applied during the juvenile period, and we hypothesized that those effects would carry over into adulthood, potentially affecting locomotor behavior [36]. To address these questions, we used HR and C male mice from all replicate lines of generation 76 of the HR animal model. During the juvenile period (3–6 weeks post birth), we administered either standard diet or WD and altered voluntary exercise by granting wheel access or not. We tested juveniles at 6 weeks for SPA, caloric intake, and body composition. After a washout period of eight weeks (equivalent to ~6 human years), we then tested adults for VE, SPA, and related traits.

2. Materials and methods

2.1. Experimental mice

Starting in 1993, four replicate lines of house mice were bred in an ongoing selection experiment for high voluntary wheel running (HR lines), based on the number of wheel revolutions on days five and six of six days of access to Wahman-type activity wheels (1.12-meter circumference) as young adults [75]. The experiment began with a population of 224 mice from the outbred Hsd:ICR strain, which was randomly mated for two generations before being randomly partitioned into eight lines. Four of these were bred randomly as Control (C) lines to the four HR lines. The current experiment used a subset of mice from generation 76. All experiments followed University of California Riverside IACUC guidelines.

2.2. Early-life diet and exercise manipulation

In this experiment, 196 male mice (half HR and half C) were weaned individually into standard cages at 3 weeks of age and placed in one of four treatment groups for 3 weeks until sexual maturity at 6 weeks of age (Fig. 1). Only males were studied due to resource constraint (e.g., the number of wheels available) and to avoid complications caused by possible estrus-cycle effects on some of the outcome variables. Half of the mice from each line were given "Western" diet (WD: Harlan Teklad TD.88137, 42% kJ from fat [anhydrous milk], 42.7% kJ from carbohydrates [sucrose and cornstarch], 15.2% kJ from protein [casein], 34.1% added sucrose by weight) and the other half were given 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]). According to Teklad/Envigo, the protein sources are very different for these two diets, so the amino acid profiles may be different as well, but both should meet all of the estimated requirements for amino acids for rodents (Derek Martin in email of 8 Feb. 2021). See Meek et al. [84] for more nutritional information on the Western and standard mouse chow.

Within each diet group, half of the animals from each line occupied cages attached to activity wheels (see above). Body mass, food consumption, and body composition were measured every seven days after weaning until mice were 6 weeks old. Photoperiod was 12:12, with lights on at 07:00 h.

2.3. Adult testing

Beginning at 6 weeks of age, all mice were individually housed with SD and without wheel access for an additional 8 weeks. At 14 weeks of age, testing of VO_2max, open-field behavior, WD and sucrose preference began. At 18 weeks of age, blood lipid levels were measured, and all adult mice were given access to wheels for 2 weeks to measure VE. Adult SPA was again measured for the subset of mice that did not have access to wheels as juveniles. Mice were euthanized and tissues collected at approximately 20 weeks of age.

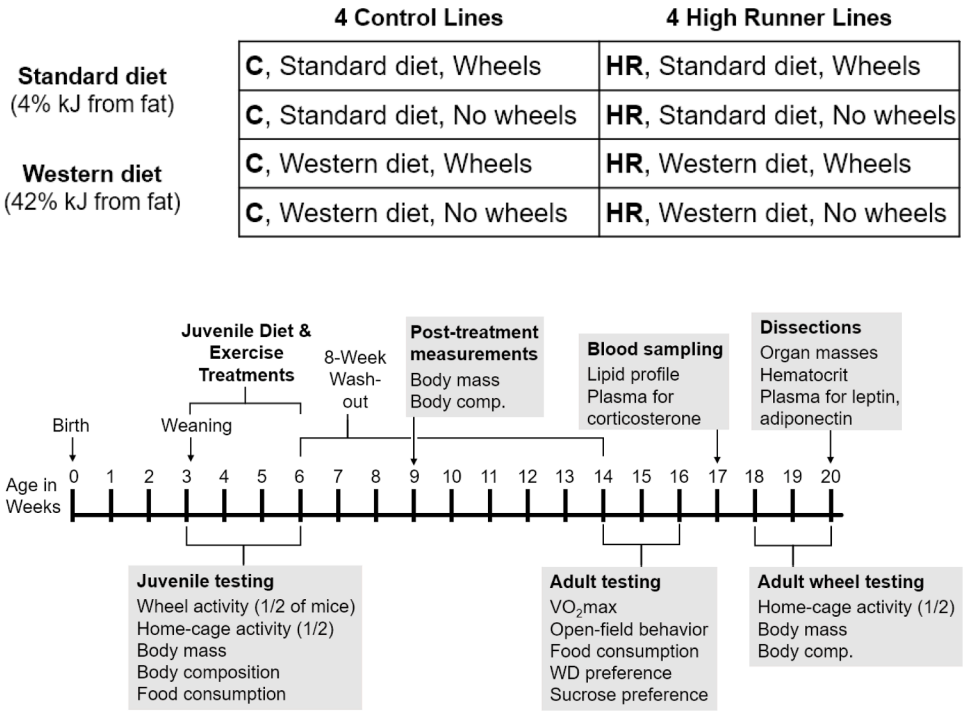

Fig. 1. Experimental timeline of events starting with the first births of generation 76 mice. Of these mice, 192 males were used for the present experiment. Three-week-long early-life diet and exercise manipulation began immediately after weaning and was followed by an 8-week washout period, during which mice were individually housed without wheels and given standard diet. At the end of washout, all mice were tested. Note that corticosterone was measured in plasma taken before wheel running, whereas leptin and adiponectin were measured in terminal blood samples collected at dissections. WD = Western diet. Home-cage activity is an indicator of spontaneous physical activity (SPA).

For each day of wheel testing (3 weeks as juveniles and 2 weeks as adults), we recorded revolutions in each 1-minute interval over a period of 23 h. This allowed computation of the total distance run, the number of 1-minute intervals with at least one revolution (a measure of the daily running duration), and the mean running speed (distance/intervals), as well as the maximum speed in any 1-minute interval [87]. We also computed weekly means for these measures. Wheel freeness (an inverse measure of how difficult it is to turn the wheel) was used as a covariate in all analyses of wheel running (e.g., [87]).

2.3.1. Home-cage activity

SPA was measured as home-cage activity. Mice were housed in home cages fitted with a passive infrared sensor (Talon TL-Xpress-A; Crow Electronics, Fort Lee, New Jersey, USA), protected within wire mesh, as in previous studies [86,87]. The sensors were connected to a computer with custom activity-recording software (developed by M. A. Chappell) via a digital I/O board (ICS 2313; ICS Electronics, Pleasanton, CA, USA). The sensors recorded activity three times per second and a mean value between 0 (no movement detected) and 1 (movement detected) was calculated for each minute over 23 h-periods of measurement. All analyses of SPA data used a measure of sensor sensitivity as a covariate [86,87]. Juvenile and adult SPA could only be measured in mice that were not given wheel access during the juvenile treatment stage.

2.3.2. Body composition

Whole-animal fat, lean, free water, and total water masses were measured by restraining each mouse within a translucent tube before insertion into an EchoMRI-100 (Echo Medical Systems, Houston, TX, USA) for scanning. This procedure lasted approximately 1–2 min per mouse and did not require sedation or anesthesia. Body composition was analyzed at weaning, after each of the three weeks of juvenile exposure, and again at 9 weeks of age, prior to adult wheel testing.

2.3.3. Caloric intake

Food consumption was measured by the change in food hopper weight each week during early-life treatment (when both SD and WD were used) and during adult wheel testing (when all mice received SD), taking care to note wasted or shredded food [88]. Weekly food consumption was converted from grams to caloric intake, taking the caloric content of SD and WD into account (14 and 19 kJ per gram, respectively; [84]).

2.3.4. Maximal aerobic capacity

To measure VO_2max, mice were subjected to forced exercise within a 900 mL enclosed mouse wheel approximately 15 cm in diameter [89,90]. Each mouse was run for approximately 5 min. while researchers manually accelerated the wheel. Duplicate trials were conducted, allowing a day of rest between each trial. Air was pumped into the enclosed metabolic chamber at a rate of 2000 mL per min, and the concentration of O_2 in excurrent air was measured by an oxygen analyzer (S-3A Applied Electrochemistry, Inc., Sunnyvale, CA). We then calculated oxygen consumption as the difference in O_2 concentration between reference air (pumped through the wheel before the mouse was placed inside) and sampled air (while the mouse was inside). Following previous studies [71,89], VO_2max was taken as the highest minute of oxygen consumption during either trial, as calculated with LabHelper software (Warthog Systems, www.warthog.ucr.edu). Trials that did not evidence a plateau in O_2 with increasing wheel speed were excluded.

Researchers subjectively assessed mouse cooperation during each trial and tiredness after each trial to assess the quality of the trial. Cooperation was scored between 1, least cooperative (the mouse made repeated attempts to breathe through the chamber aperture and/or resisted moving in the direction of motion) and 5, most cooperative (the mouse was least interested in the aperture and focused mostly on running with the direction of rotation). Tiredness was scored between 1, least exhausted after the trial, to 3, most exhausted. Tiredness (1–3 scale) was scored based on long it took for the mouse to begin moving about the respiration chamber after the end of forced exercise (1 = 1 s or less; 3 = 5 s or more). The identity of the investigator scoring each trial was recorded, so that potential differences in scoring could be accounted for. Trials were excluded from analyses if cooperation scores were less than 3 or tiredness scores were less than 2, as values this low likely correspond to trials in which VO_2max would not be attained.

2.3.5. Preference for Western diet and sucrose solution

At approximately 17 weeks of age, adult mice were presented in their

home cage with partitioned food hoppers containing WD and SD for 24 h (13:00–13:00 h). Consumption of each diet was measured as the change in hopper weight across the test period, with due allowance for spillage and wasting [88]. The next day, mice were given a similar choice between water and sucrose-water (13:00–13:00 h) (10.5% sucrose; Fisher Scientific Certified ACS Grade). WD preference was calculated by caloric conversion: WD kJ / (WD kJ + SD kJ) [84,91]. Sucrose preference was calculated similarly as volume of sucrose-water / (volume of sucrose--water + volume of water).

2.3.6. Open-field behavior

Between 15 and 16 weeks of age, mice were tested for open-field behavior [92] on reversed photoperiod (at approximately 07:00–18:00 h). Each mouse was placed in the center of an arena (1 m^2) surrounded by 0.5 m black Trovicel walls under low-light conditions [93]. The mouse was recorded for 5 min with a Logitech HD C525 Webcam placed above the arena. Videos were assessed with TopScan LITE software (Clever Sys Inc., Reston, VA). The amount of urine (scored as 0, 1, 2) and the number of fecal pellets left by the mouse during the test were recorded. The arena was cleaned with warm, soapy water and dried between each trial. Analyses of time spent near the arena walls were done on each min of the trials, using time of day as a covariate.

2.3.7. Blood lipid profile

At 17 weeks of age, food was removed at 07:00 h and blood samples were taken from the submandibular vein using sterile, single-use lancets under isoflurane anesthesia at 13:00–19:00 h [94]. Total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, triglycerides, and glucose (GLU) in whole blood, as well as TC/HDL (total cholesterol/HDL cholesterol) ratio and estimates of low-density lipoprotein (LDL) and non-HDL cholesterol were measured and calculated by an Abbott Cholestech LDX™ Analyzer. Time of day and bleed delay time (elapsed time from disturbing the mouse to collecting a blood sample) were used as covariates in statistical analyses.

2.3.8. Dissections and plasma hormone concentrations

Mice were removed from wheel access for one day then euthanized via decapitation. Trunk blood was immediately collected into heparinized containers, and organs (brain, heart ventricles, spleen, liver, triceps surae muscles, and fat pads) were dissected and weighed to 0.0001 g. Plasma samples were prepared and preserved as instructed by the assay kits described below.

Terminal plasma leptin was measured in duplicate using a Crystal Chem enzyme-linked immunosorbent assay (ELISA) kit (Mouse Leptin Assay Catalog #90,030) without dilution. Absorbances were read at 450 nm in an EPOCH2 microplate reader using GEN5 2.07 reading software (microplate and reading software: BioTek Instruments, Winooski, VT, USA). Terminal plasma adiponectin was measured in duplicate with an AssayPro ELISA kit (Mouse Adiponectin ACRP30 Catalog #EMA 2500–1), using a 400-fold dilution. Prior to dissection, plasma was collected from the caudal vein at 17 weeks of age (Fig. 1) specifically to measure corticosterone concentration in duplicate with an Arbor Assays ELISA kit (Corticosterone EIA kit Catalog #K014-H1), using a 100-fold dilution. All assay plates were read in triplicate.

2.3.9. Statistical analysis

Data were analyzed as mixed models in SAS 9.1.3 (SAS Institute, Cary, NC, USA) Procedure Mixed, with REML estimation and Type III Tests of Fixed Effects. Depending on the trait analyzed, body mass, age, wheel freeness, and home-cage sensor sensitivity were used as covariates. Linetype (HR vs. C), diet, and wheel access were fixed effects, while replicate lines were a random effect nested within linetype. Effects of linetype, diet, and wheel access, as well as their interactions, were tested with 1 and 6 degrees of freedom. Modeling the aforementioned random effects occasionally resulted in covariance parameter estimates of zero for some of the interactive random terms. In these cases, the interactive random terms were removed, starting with higher-order terms. The line within linetype random term was never removed. Once analyses returned non-zero covariance parameter estimates, outliers were then removed when the standardized residual exceeded ~3.

An additional fixed effect, termed mini-muscle, was included in all statistical analyses. The original base population of the outbred strain had a mini-muscle phenotype, which is caused by a Mendelian recessive allele that is a novel intronic single nucleotide polymorphism in the *Myosin heavy polypeptide 4* gene [95]. The allele started at a frequency of ~7% in the base population and was under positive selection in the HR lines, eventually going to fixation in one HR line while remaining polymorphic in another [96–99]. The mini-muscle phenotype is characterized by a 50% reduction in the triceps surae muscle and total hindlimb muscle mass – due largely to reduced type IIb muscle fibers [100,101]. The effect of mini-muscle phenotype is reported in tables of statistical results but, for simplicity and as it is not a primary focus of the present study, not mentioned in the Results. In Discussion, we summarize results of the mini-muscle phenotype.

Leptin and adiponectin concentrations were analyzed with covariates of fat mass (as measured by MRI). To remove outliers from plasma hormone data, we tested for variation due to multiple reads of the assay plates and variation between duplicates. SAS Procedure Mixed was used with Mouse ID as the independent variable and sample duplicate nested within Mouse ID and read triplicate nested within sample duplicate as random effects. Corticosterone samples with bleed delay time of 3 min or more were excluded from analysis ($n = 10$). As described above, outliers were removed when the standardized residual exceeded ~3.

To investigate any possible effects of litter sex ratio at weaning, we used sex ratio as a covariate in many relevant preliminary tests (e.g., juvenile and adult wheel running distance, body mass and composition, maximal aerobic capacity, etc.). The effect of sex ratio was never statistically significant (results not shown), so final models did not include it as a covariate.

3. Results

Juvenile exposure to a Western diet and/or voluntary exercise had numerous effects on juvenile and adult wheel-running behavior, body mass and fat composition, and other behavioral and physiological traits, some in an interactive genotype-by-environment manner.

3.1. Juvenile wheel running and spontaneous physical activity (during treatment)

During the first week of treatment, WD increased the distance (i.e., number of revolutions) run by 29% in C mice and by 48% in HR mice (Fig. 2A; diet $p < 0.0001$; diet × linetype $p = 0.0072$). During the second week, mice on WD ran 25% more revolutions than those on SD (diet $p = 0.0188$) and HR mice ran 160% more than C mice (linetype $p = 0.0320$), with no interaction. Diet effects disappeared completely by the third week, and HR mice ran more than 3-fold more revolutions per day than C mice ($p = 0.0006$). See Fig. 2B–D and Supplemental Table 1 for results on duration, average speed, and maximum speed.

Home-cage activity was only recorded for mice housed without wheels, and only during the third week of exposures. Diet did not affect juvenile SPA ($p = 0.5734$); however, HR mice were significantly more active in the home-cage than C mice ($p = 0.0036$) (Supplemental Table 1).

3.2. Adult wheel running and spontaneous physical activity

In the first week of adult testing, HR mice ran 3.5-fold more distance than C mice (Fig. 3; $p = 0.0008$). Early-life exercise increased wheel running distance among C mice, but not HR mice (exercise × linetype $p = 0.0512$). Early-life WD increased distance for HR mice, but not for C mice (diet × linetype $p = 0.0091$). In the second week, HR mice ran 3.3-

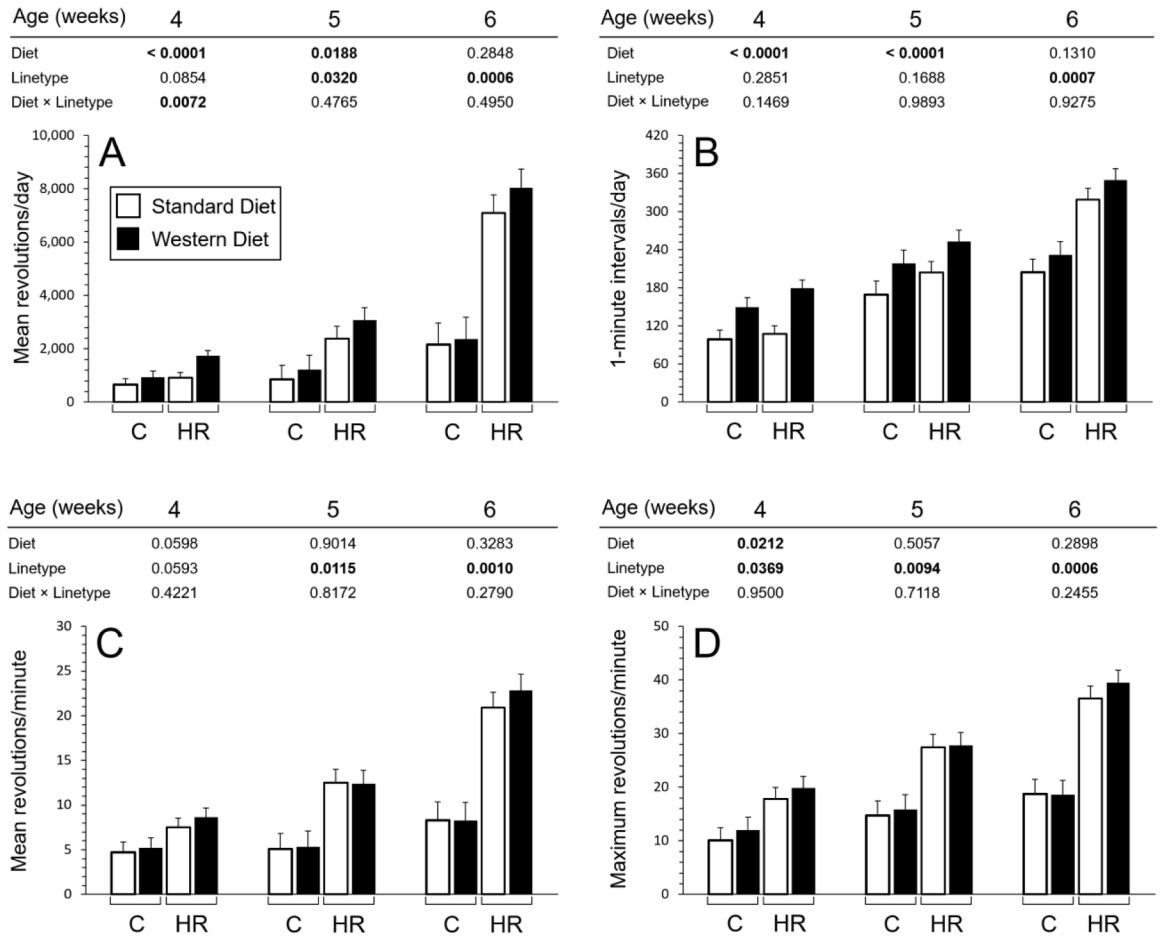

Fig. 2. Juvenile wheel running of male mice during the 3-week treatment period, shown as least squares means and standard errors with accompanying p-values from SAS Procedure Mixed. Note the data shown are from the experimental group given access to wheels (see Fig. 1). A) Mean wheel revolutions per day (circumference 1.12 m), B) duration of daily running, C) mean revolutions per minute, D) maximum revolutions per minute. Above each week (set of four bars) of measurements are tables of main and interactive effects ($p < 0.05$ in bold font). See Supplemental Table 1 for additional statistical details.

fold more than C mice ($p < 0.0001$; Supplemental Table 1) and early-life WD exposure significantly increased average daily running distance of HR mice ($p = 0.0186$), but not C ($p = 0.8914$; diet × linetype interaction $p = 0.0534$).

During the first week, daily running duration, mean speed, and maximum speed averaged greater for HR mice (all $p \leq 0.0251$). However, significant diet × linetype interactions occurred ($p = 0.0146$, 0.0414, and 0.0761; Fig. 3B, C, and D, respectively), whereby early-life WD decreased these measures among C mice but increased them among HR. Early-life wheel access increased mean and maximum speeds, but not running duration (all $p \leq 0.0086$). See Supplemental Table 1 for results from the second week of adult wheel running.

As noted above, only mice that did not have wheel access during the juvenile period were measured. SPA of adult mice housed with wheel access was unaffected by juvenile diet ($p = 0.4535$) and did not differ between HR and C mice ($p = 0.2218$), with no diet × linetype interaction ($p = 0.3496$ for first week; results for week two in Supplemental Table 1).

3.3. Body mass and composition

3.3.1. Body mass

Body mass increased throughout the experiment for all four experimental groups for both HR and C lines, until finally declining during the two weeks of adult wheel testing for all groups (Fig. 4A, B). The change in body mass from the end of juvenile exposures (9 weeks of age) to the start of adult wheel testing (18 weeks of age) was not affected by juvenile treatments, with or without body mass at 9 weeks of age as a covariate. The change in body mass across the two weeks of adult wheel testing was influenced by a three-way interaction of juvenile exercise, juvenile diet, and linetype ($p = 0.0059$). Mice from the HR lines were significantly smaller than C mice at all time points, including at weaning (all $p \leq 0.0020$).

3.3.2. Lean mass

Body lean mass increased throughout the experiment (Fig. 4C, D). Adult lean mass did not change significantly across two weeks of wheel testing. Early-life WD increased lean mass a week after treatment ended ($p = 0.0383$), and this effect persisted through washout into adulthood ($p = 0.0316$). Access to wheels during the juvenile period increased lean mass after the third week of treatments ($p = 0.0104$). HR mice were consistently leaner than C mice during early life and into adulthood (all $p < 0.05$, Supplemental Table 1).

3.3.3. Fat mass

During each week of juvenile treatment, WD significantly increased body fat in all groups (Fig. 4E, F; all three $p \leq 0.0001$, with lean mass as a covariate), with C mice on WD having the sharpest rise (compared to HR mice on WD) in body fat after 3 weeks of juvenile treatment (diet × linetype $p = 0.0099$). The apparent increase in relative body fat between 9 and 18 weeks of age was not statistically significant for any group, with no significant effect of linetype or early-life treatment. After

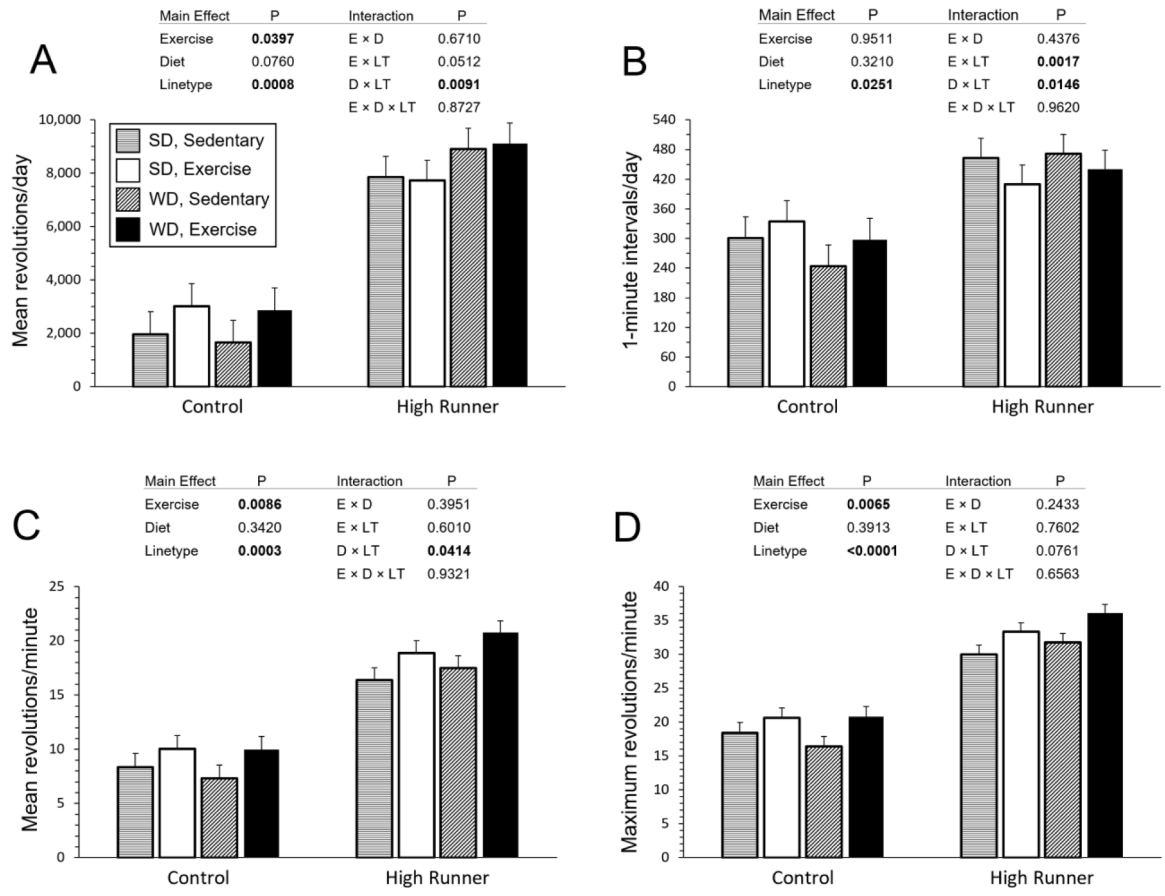

Fig. 3. Adult wheel running during days 1–7 of a 2-week testing period. Values are least squares means and standard errors and accompanying p-values from SAS Procedure Mixed. A) Mean wheel revolutions per day (circumference 1.12 m), B) duration of daily running, C) mean revolutions per minute, D) maximum revolutions per minute. Above each measurement are tables of main and interactive effects ($p < 0.05$ in bold font). SD = standard diet; WD = Western diet; E = exercise; D = diet; LT = linetype. Values for the second week of adult testing can be seen in Supplemental Table 1.

two weeks of adult wheel testing, relative body fat was significantly reduced in all groups (all $p \leq 0.0138$). At the end of adult wheel testing, relative fat mass had a three-way interaction (exercise × diet × linetype $p = 0.0224$).

3.4. Caloric intake

During the first week of early-life treatment, WD increased mean daily caloric intake by ~21% (Fig. 5; diet $p < 0.0001$). Among SD mice, those with wheels consumed significantly more calories than the sedentary group; however, for WD mice, those with wheel access consumed significantly fewer calories than the sedentary group (exercise × diet $p < 0.0001$). During the second week of treatment, both exercise ($p = 0.0024$) and diet ($p = 0.0009$) increased caloric intake, with no interactions. During the third week of treatment, mice with wheels consumed more ($p < 0.0001$), and the effect was somewhat greater for HR mice (diet × linetype $p = 0.0881$).

In the first week of adult testing, the early-life treatments did not affect caloric intake. However, in the second week of adult testing caloric intake was decreased by early-life exercise, and the effect was greater among HR mice (Fig. 6; exercise × linetype $p = 0.0126$). HR mice consumed more calories than C mice during both the first (Fig. 6; $p = 0.0276$) and second ($p = 0.0178$) weeks of adult wheel testing.

3.5. Maximum aerobic capacity

With body mass as a covariate, HR mice had greater VO_2max than C mice (Fig. 7; $p = 0.0012$). Neither early-life exercise, nor diet had any effect on VO_2max.

3.6. Preference for Western diet and sucrose-water

All experimental groups preferred WD to SD (see Supplemental Table 1). Early-life WD increased adult preference for Western diet in all groups, and a three-way interaction indicated that the magnitude of this effect depended on linetype and early-life exercise (see Supplemental Table 1; $p = 0.0578$). All groups preferred sucrose-water to regular water, but this preference was not affected by linetype or either early-life treatment.

3.7. Open-field behavior

Early-life exercise reduced the time spent near the arena walls during each of the five minutes of the open-field test for all groups (all $p < 0.05$). Summing across all five minutes also showed a 3-way interaction (exercise × diet × linetype $P = 0.0432$), indicating that the magnitude of the effect varied somewhat among groups. We found no statistical effects on distance moved, except for a linetype effect during minute 5 ($p = 0.0494$; HR > C by ~12%) and an exercise × diet interaction for minute 3 ($p = 0.0412$). We found no statistical effects on open-field defecation or defecation plus urination (Supplemental Table 1).

3.8. Blood lipid profiles

Using bleed delay time and time of day as covariates, adult levels of triglyceride and total cholesterol were unaffected by early-life

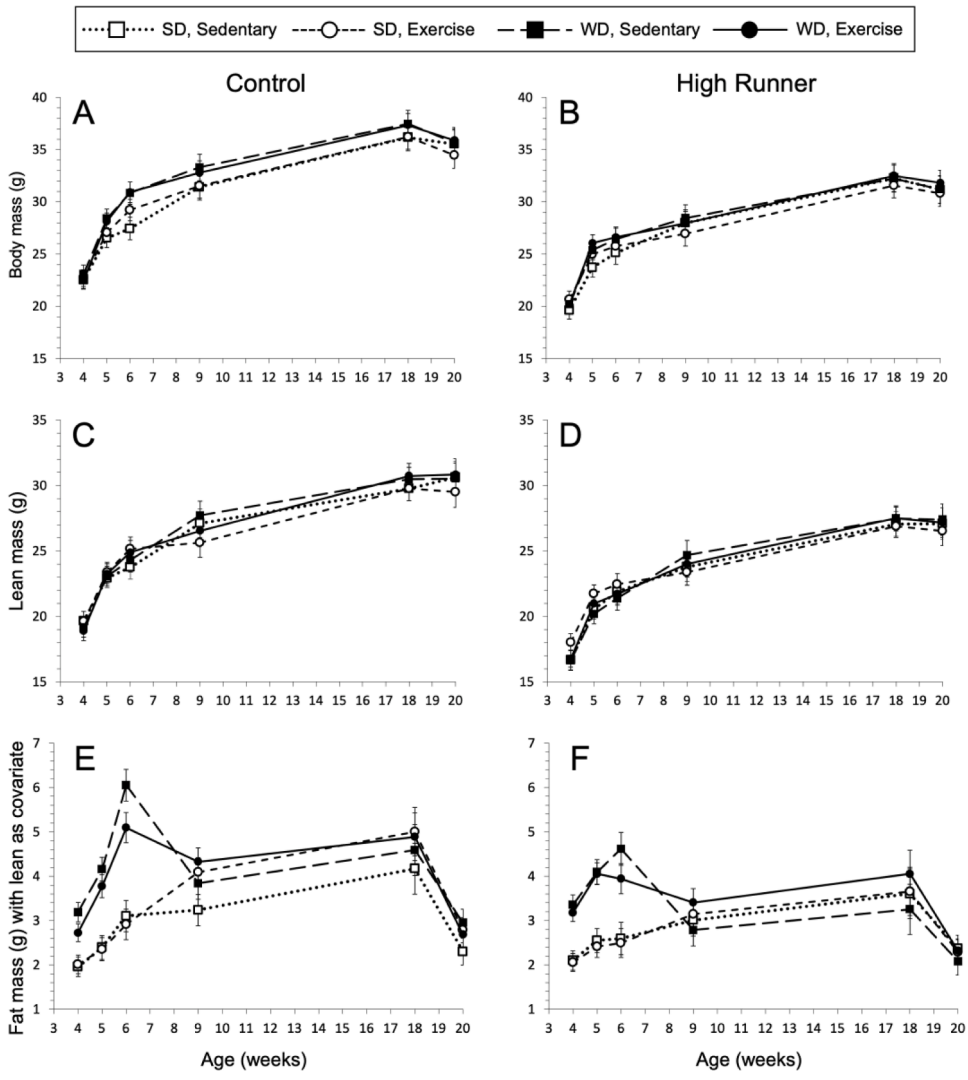

Fig. 4. Total body mass, lean mass, and fat mass. Values are least squares means and standard errors from SAS Procedure Mixed. Note that body composition (lean and fat mass) was not measured during weaning (week 3); however, HR mice at weaning weighed 12.61% less than C mice ($p = 0.0278$). Fat mass was analyzed using lean mass as a covariate. See Supplemental Table 1 for additional statistical details. SD = standard diet, WD = Western diet, Sedentary = housed without wheels as juveniles, Exercise = housed with wheels as juveniles.

treatments. Fasted blood glucose levels were increased by early-life exercise (Supplemental Table 1; $p = 0.0140$).

3.9. Circulating levels of leptin, adiponectin, and corticosterone

HR mice had approximately 17% greater corticosterone concentration than C mice (Supplemental Table 1; $p = 0.0068$). Among mice exposed to an early-life SD, early-life exercise decreased corticosterone concentration for C mice but increased it for HR (Supplemental Table 1; exercise × diet × linetype = 0.0259). Using fat mass as a covariate, early-life exercise treatment increased adult leptin concentration (Supplemental Table 1; $p = 0.0173$), while adiponectin concentration was not affected by linetype or either early-life treatment.

3.10. Organ masses

Early-life exercise increased adult triceps surae, subdermal fat pad, and brain mass, but decreased heart ventricle mass (Fig. 8; all $p \leq 0.0170$). Reproductive fat pad and spleen masses were unaffected by either early-life treatment or genetic background. The hearts and brains of HR mice were heavier than those of C mice (all $p \leq 0.0270$).

4. Discussion

Physical activity should be a cornerstone of effective prevention and treatment strategies for obesity and its comorbidities [102–108]. Given the risk of overweight children developing into unhealthy, overweight adults [109–113], it is important to attempt to identify early-life factors that might have lasting, positive effects on adult physical activity [114]. Implementation of such early-life factors could be much more efficient than waiting until metabolic, cardiovascular, and other disorders appear years later [115,116].

The present study is one of the few to examine the possibility of lingering early-life effects of diet and/or physical activity after a substantial washout period with no additional treatment(s). In a full factorial experiment using mice, we tested whether Western diet and/or access to wheels during the juvenile period would: (1) affect juvenile physical activity, caloric intake or body composition; (2) have long-lasting effects into adulthood, following a substantial washout period; and (3) have interactive effects with each other and/or with genetic background. To our knowledge, no other experimental studies have integrated all three factors. We found a number of acute effects of diet and exercise manipulation in juvenile male mice as well as many longer-lasting effects in adults. In addition, some early-life effects interacted with genetic linetype (selectively bred High Runner vs. non-selected Control lines of mice). Our results have implications for understanding pre-pubertal environmental effects in humans, including the complex interactions that result in human obesity and its negative health consequences.

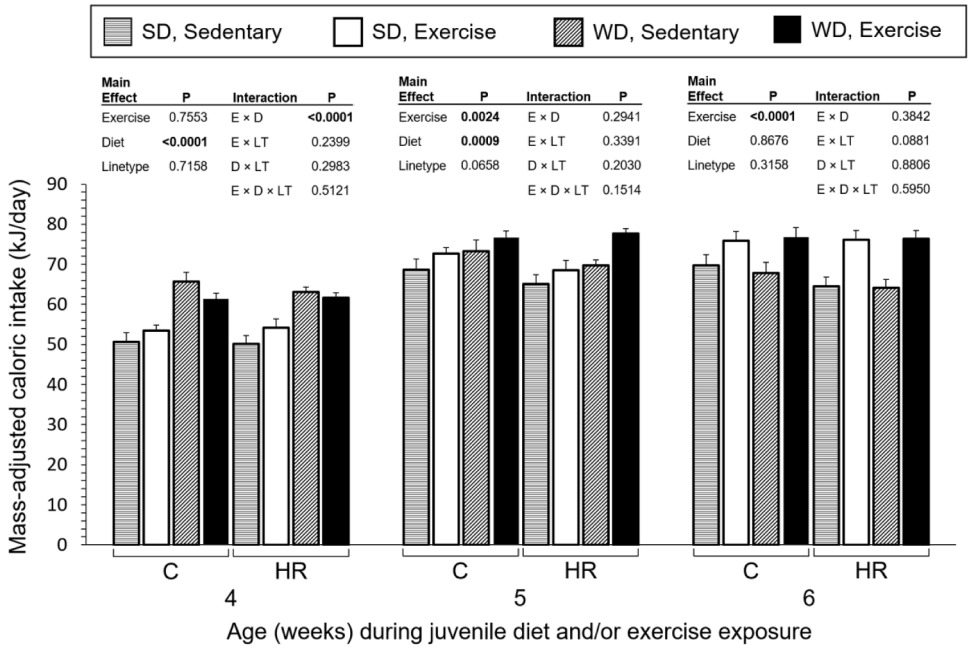

Fig. 5. Weekly mass-adjusted juvenile caloric intake in response to juvenile diet and/or exercise treatment. Values are least squares means and standard errors and accompanying p-values from SAS Procedure Mixed. Above each week are tables of main and interactive effects ($p < 0.05$ in bold font). See Supplemental Table 1 for additional statistical details.

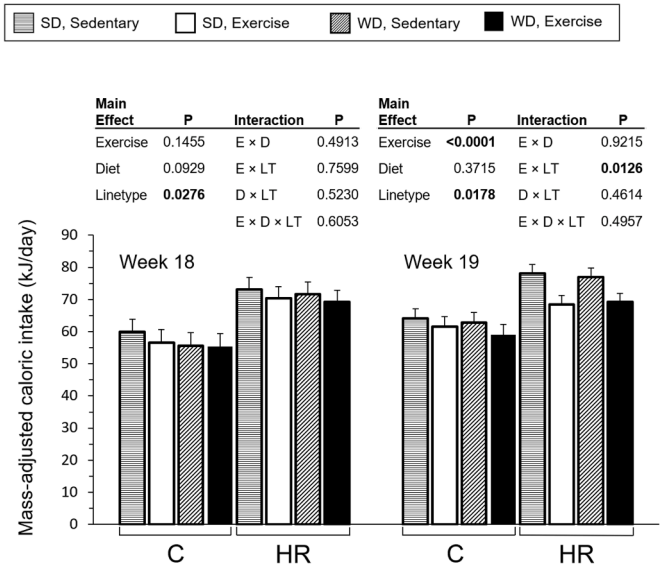

Fig. 6. Adult caloric intake with body mass as a covariate. Values are least squares means and standard errors and accompanying p-values from SAS Procedure Mixed. Above each week are tables of main and interactive effects ($p < 0.05$ in bold font). See Supplemental Table 1 for additional statistical details.

4.1. *Immediate and long-lasting effects of early-life Western diet and exercise*

4.1.1. *Wheel running*

Western diet (WD) increased voluntary exercise (total distance run) during weeks 4 and 5 of the initial period of juvenile exposure (Fig. 2) and also when adults were retested (Fig. 3), mainly through increased running duration. This effect was greater for HR mice, and in a previous study was observed only in HR mice [84]. Together, these results suggest that inherently athletic individuals and/or those who are more physically active might gain a greater benefit from high-energy diets.

Juvenile exercise increased adult running distance for C mice only (Fig. 3: exercise × linetype $p = 0.0512$). In contrast, Acosta et al. [86] found that early-life wheel access increased distance run in both HR and C mice. This difference might relate to previously an observed seasonal variation in wheel running (see Fig. S4 in [74]), as the mice in Acosta et al. [86] were weaned in June whereas ours were weaned in October. In both studies, early-life wheel effects on adult running distance were transient in that they became statistically nonsignificant after the first week of adult testing (Supplemental Table 1).

Although the positive effects of juvenile Western diet and exercise that we observed lasted for only one week, this translates to ~9 months for humans [117,118]. As these sorts of early-life environmental factors can be manipulated relatively easily (e.g., through school cafeterias or youth sports programs), they may be an efficacious tool in the fight against the obesity epidemic and adult sedentary behavior, although they likely need to be combined with reinforcers during adulthood [119–121].

4.1.2. *Body mass and composition*

During the juvenile period, male mice given access to wheels did not show the expected reduction in body mass, compared to juveniles without wheel access (e.g. for male mice see [86,122,123]). In fact, after three weeks of early-life exposure, mice with wheels had *increased* body mass compared to those without wheels ($p = 0.0415$); this difference was related to increased absolute lean mass ($p = 0.0104$) and accompanied by slightly reduced body fat ($p = 0.0936$ or $p = 0.0552$ with lean mass as a covariate).

At the start of adult wheel testing, early-life wheel access had no significant effect on body mass (consistent with [86]), lean mass or fat mass (see Fig. 4 and Supplemental Table 1), although HR mice weighed significantly less and had lower lean mass as compared with C mice. To our knowledge, no other study has reported effects of early-life exercise on adult body mass after a substantial washout period.

All treatment groups lost body mass across the two weeks of adult wheel testing, but the magnitude of these reductions differed among groups (exercise × diet × linetype interaction $p = 0.0059$). With respect to body composition, none of the eight experimental groups lost a statistically significant amount of lean mass, whereas all of them lost

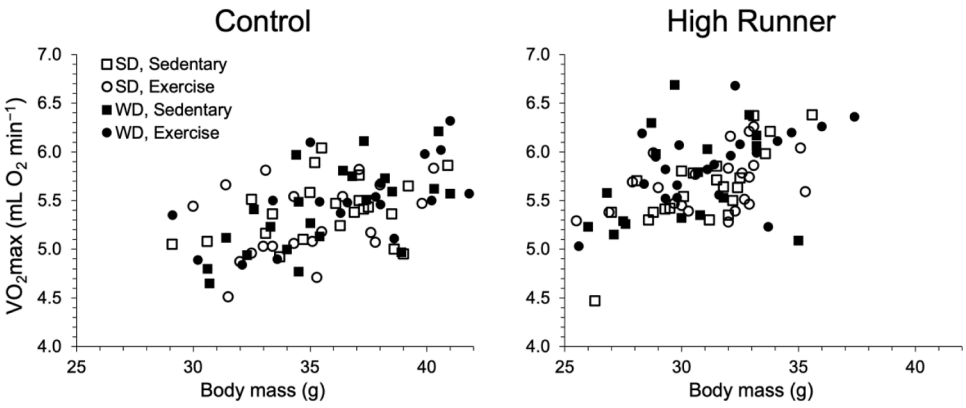

Fig. 7. Maximal oxygen consumption (VO_2max) measured during forced exercise (see Methods 2.3.4). Body mass was a significant predictor of VO_2max ($p < 0.0001$), mice from High Runner lines had higher VO_2max than those from non-selected Control lines ($p = 0.0019$), and individuals with the mini-muscle phenotype (not shown with a separate symbol) had higher values than normal-muscled mice ($p = 0.0271$), with no effect of either early-life treatment and no interactions (see Supplemental Table 1 for additional statistical details).

significant fat mass (see Supplemental Table 1). Consistent with a previous study that used a 6-day wheel test, HR lines lost less fat mass than C mice during adult wheel testing (see Supplemental Table 1) [124], indicating that the regulation of body weight when faced with acute exercise varies with genetic background.

Although WD consistently increased fat mass during the three weeks of early-life exposure, simultaneous exercise partially protected mice from this increase (though it was not eliminated completely (exercise x diet $p = 0.0170$ for Week 1, $p = 0.5136$ for Week 2, and $p = 0.0355$ for Week 3)). This effect mostly faded by the end of the 8-week washout, during which all mice received SD. Early-life WD increased adult lean mass ($p = 0.0316$), but the effect disappeared after two weeks of adult exercise ($p = 0.0960$).

4.1.3. Caloric intake

We expected that body mass-adjusted caloric intake would increase with wheel access [86,87,125]. Surprisingly, in the first week of juvenile treatments, early-life wheel access decreased caloric intake for mice feeding on WD and increased it for mice feeding on SD (Fig. 5). During this first week, mice are experiencing multiple environmental changes (novel exposures), including not only diet and exercise opportunity, but also single housing, which affects social behavior and thermoregulation, so their normal homeostatic mechanisms (both physiological and neurobiological) may not yet have adjusted. During the second and third weeks, however, wheel access had the expected positive effect on caloric intake and did not interact with diet or linetype. In any case, the neural and endocrine mechanisms that underlie homeostatic responses to novel (acute) versus longer-term (chronic) environmental changes deserve further study.

During the first week of adult wheel testing, HR mice had greater mass-adjusted caloric intake than C mice, consistent with previous studies (e.g., [86,87,124]), with no statistical effects of either early-life WD ($p = 0.0929$) or exercise (Fig. 6). During the second week of adult testing, an effect of early-life exercise emerged, in which caloric intake was decreased in HR mice, but not in C mice (exercise × linetype $p = 0.0126$). When we added distance run as a covariate, to account for the greater running by HR mice, the interaction disappeared, and the effect of early-life exercise was negative for both HR and C mice (Supplemental Table 1). Given that early-life exercise increased adult wheel running in both HR and C mice, and that HR mice ran much more than C, this effect suggests an alteration in digestive or metabolic efficiency, or perhaps in the regulation of body weight, although the loss in body mass across the two weeks of adult wheel access did not show any statistically significant interactive effects (Supplemental Table 1). In any case, early-life exercise exposure combined with adult exercise had a dampening effect on caloric intake, which could have important implications for human health [126–128].

4.1.4. Western diet and sucrose preference

As adults, almost all mice preferred the WD to standard diet when given 24-hour access to both diets, as is commonly reported (e.g., [91]). On average, however, those given WD as juveniles had a significantly stronger preference for WD than those given juvenile SD (Supplemental Table 1). Although WD is high in sugar and fat, we did not find any statistically significant effects on adult sucrose preference, suggesting that familiarity with the taste of the fatty component of WD may be driving the diet preference. Additionally, a three-way interaction effect on WD preference was found (exercise × diet × linetype $p = 0.0578$), indicating some effect of genetic background. Although few rodent studies are available (e.g., [66]), various human studies indicate that early-life factors can affect adult dietary preferences (e.g., [54,129]).

4.1.5. Open-field behavior

Early-life wheel access generally reduced adult anxiety-like behavior (i.e., less time spent near walls in the open field), although the strength of the effect varied among groups (exercise × diet × linetype $p = 0.0432$). In principle, effects on anxiety-like behavior might be mediated by plasma corticosterone levels [130,131]. As noted in the next section, adult plasma corticosterone levels also showed a 3-way interaction (exercise × diet × linetype $p = 0.0259$). The patterns for these 3-way interactions were somewhat different, but the correlation of the eight group means for time spent near walls (over all 5 min, squared) and plasma corticosterone (log-transformed) was positive (Pearson's $r = 0.546$, $p = 0.1615$), suggesting that corticosterone might have played some role in mediating effects of early-life wheel access on anxiety-like behavior. The reduction in anxiety-like behaviors cannot be attributed to effects of early-life wheel access on activity levels because distance moved in the open-field test was not affected in a way that paralleled time spent near walls. An interesting direction for future studies would be to evaluate a possible role of the neuropeptide corticotropin-releasing hormone, which regulates corticosterone secretion, strongly influences anxiety-like behavior, and has been suggested to mediate effects of experience (stress) during the juvenile period on adult phenotypes [132–134].

4.1.6. Leptin, adiponectin, corticosterone, and blood glucose

Leptin is produced by white adipose tissue and, among other functions, regulates body weight by increasing energy expenditure and inhibiting food intake [135–137], along with a host of other effects, including an important role in glucose metabolism [138]. As expected from numerous previous studies, including those involving the HR mice [71,86,139], body fat was a strong positive predictor of adult levels of circulating leptin in the present study. In addition, early-life wheel access increased fat-adjusted leptin levels in both linetypes, whereas Acosta et al. [86] found that early-life wheel access interacted with linetype (no wheels: HR>*C*; wheels: HR<*C*). The discrepancy in these results may be explainable by Acosta et al.'s. use of visceral fat pad mass

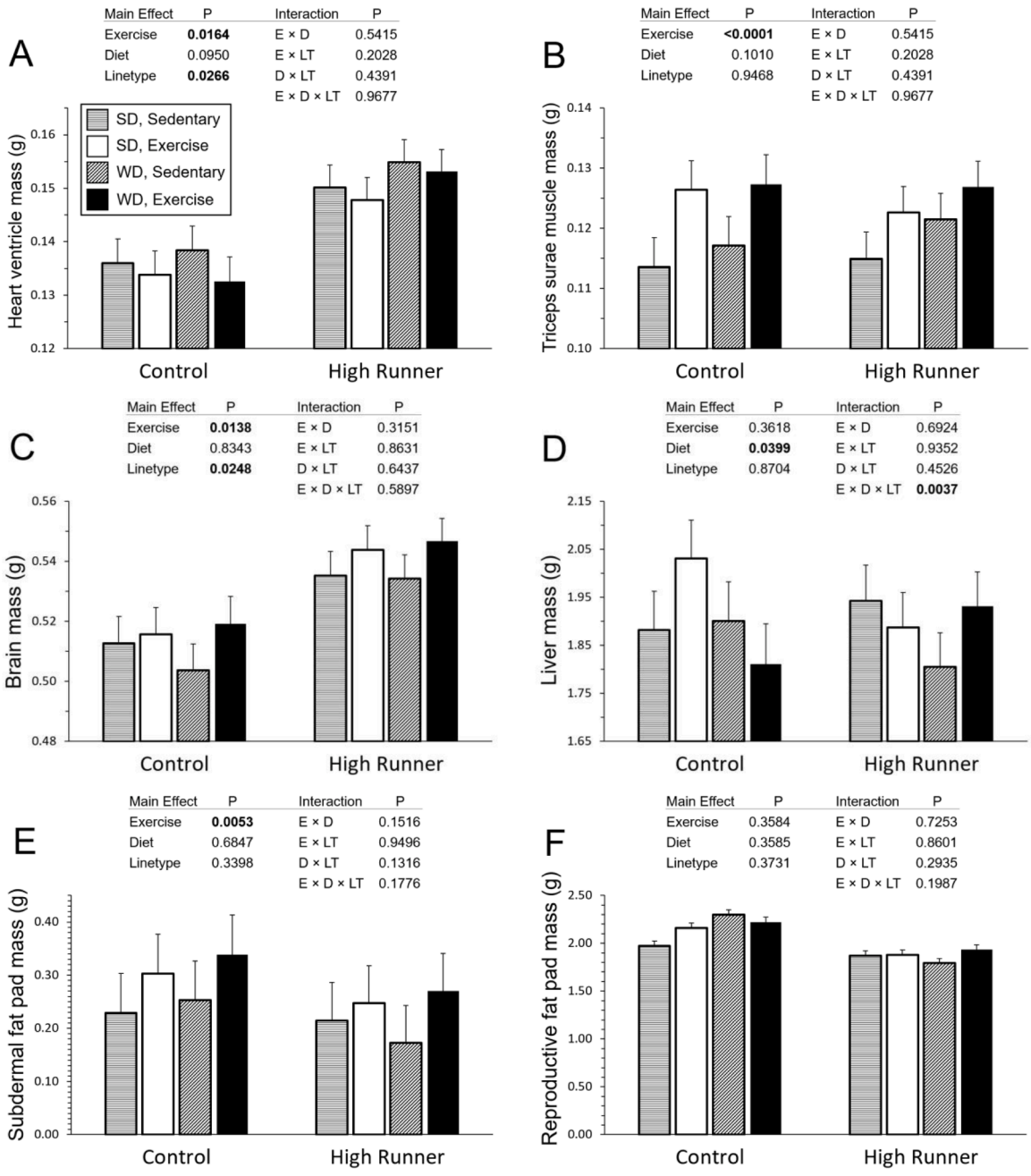

Fig. 8. Body-mass adjusted heart ventricles, triceps surae, brain, liver, and subdermal fat pad masses. Values are mass-adjusted least squares means and standard errors and accompanying p-values from SAS Procedure Mixed with body mass as a covariate. See Supplemental Table 1 for additional statistical details.

and our use of body fat measured by MRI as covariates: dissected visceral fat pads are only a subset of a mouse's leptin-producing adipocytes; however, not all body fat measured via MRI produces leptin [140]. It is also worth noting that leptin regulation in mice can be influenced by age [141]; we collected terminal serum samples when mice were 20 weeks old, whereas Acosta et al. [86] took samples at ~15 weeks.

Although some previous studies have reported higher plasma adiponectin levels in HR mice as compared with C lines [36,142,143], we did not find any statistically significant effects on adiponectin. However, adult plasma corticosterone showed a 3-way interaction (exercise × diet × linetype $p = 0.0259$), as well as a main effect of linetype ($p = 0.0068$). The selected HR lines always have elevated corticosterone levels, as has generally been found in previous studies (e.g., [144–147]; but see [71,143]). In addition, HR mice exposed to early-life wheel access and SD had much higher levels of corticosterone than any other group (Supplemental Table 1).

To our knowledge, no previous studies have tested for long-lasting effects of early-life exercise on adult blood glucose concentrations. We observed higher glucose levels in mice given juvenile wheel access than in those housed without wheels.

Perhaps this effect is related to the effects observed when humans and rodents are cycled between standard and high-fat diets (e.g., [148]). It is also possible that early-life exercise has affected glucose tolerance [149]; however, the current study did not include a glucose tolerance test, which may have been useful in addressing these questions. In any case, the early-life exercise effects on leptin noted above may be involved in the effects on blood glucose [138].

4.1.7. Organ masses

Numerous studies spanning many decades show that providing laboratory rodents with chronic wheel access leads to changes in the size of internal organs relative to body size, but few have tested for long-lasting effects of juvenile exercise alone. In a previous study of male HR and C mice, Acosta et al. [86] found no effects of early-life wheel access on visceral fat pads, heart ventricles, liver, or spleen masses. Some of our results are similar, but others differ. Heart ventricle and liver masses were affected by multiple interactions among diet, exercise, and linetype (see Fig. 8A, C). These effects could be either beneficial or detrimental, depending on the cause. For example, an increase in liver mass may be detrimental if caused primarily by WD (Fig. 8D), which often causes cirrhosis or non-alcoholic fatty liver disease ([150,151], respectively).

An apparently novel finding was the positive effect of early-life exercise on calf-muscle mass for all groups (Fig. 8B). This type of effect, if it occurs in humans, has obvious relevance for human health from early-life to advanced age [152–154].

Another notable effect of early-life exercise was an increased brain mass in all groups. Several recent studies have shown that chronic exercise can increase brain mass in rodents, even within one week [155]. To our knowledge, however, this is the first report of a juvenile effect on brain mass that persists into adulthood after a substantial washout period. Exercise is known to upregulate growth factors, including brain-derived neurotrophic factor, which may have an even larger effect during a developmental period (e.g., see [156] for rodents and [157] for humans).

4.2. Differences between high runner and non-selected control lines

The current study reaffirms many previously reported differences between the HR and C linetypes [71,76,83,86]. Average daily wheel-running distance during juvenile testing was higher for HR than C mice, due mainly to greater average running speeds, consistent with many previous studies (e.g., [84,86,123]) (Fig. 2A).

In addition, as adults, HR mice were smaller in total body mass and had leaner bodies, and (with body mass as a covariate), they had greater maximal aerobic capacity, heavier hearts and brains, and elevated circulating levels of corticosterone. Other differences, including several that involved interactions with early-life diet and/or exercise exposure, are mentioned above.

4.3. Characteristics of mini-muscle individuals

As described above (see *2.3.9. Statistical analysis*), the mini-muscle phenotype and underlying gene were an unexpected discovery in the High Runner mouse selection experiment [95,96]. In addition to their greatly reduced hindlimb muscle mass, the present study confirmed several previously reported differences between mini- and normal-muscled individuals, including heavier heart ventricles, livers, and greater VO_2max [71,77,158–160]. In addition, we found some new differences, including that mini-muscle individuals spent more time near the walls in an open-field behavior test (suggesting elevated anxiety) and increased total body fat. Future studies will be required to determine the molecular and cellular pathways through which the mini-muscle gene causes such a wide range of pleiotropic effects.

5. Limitations and concluding remarks

One limitation of the present study is that all mice were given two weeks of adult wheel access, followed by one day with the wheels removed, prior to dissection. Therefore, organ masses and circulating concentrations of adiponectin and leptin would have been affected by early-life effects, two weeks of training effects (physical conditioning), plus any acute "rebound" effects that might have occurred over ~24 h (e.g., see [161]). Any of these effects could have obscured the others. In future experiments, we intend to have multiple cohorts of subjects, such as one that is sampled prior to adult wheel testing.

Another limitation is that we studied only males. Importantly, nutrient intake in early life has been shown to have sex-specific effects on the regulation of physical activity (i.e., wheel running) in mice [162]. In an early-life experiment on the same mouse model as the current study, Hiramatsu et al. [71] observed sex differences as well (see their Fig. 8 and online supplemental Fig. 4). Moreover, given that many aspects of physical activity (including both motivation and ability), exercise physiology, body composition, and hormone profiles are sexually dimorphic in both mice [163–167] and humans [147,168,169,170–171], future studies should include both sexes.

Considering only statistically significant main effects ($p < 0.05$), we observed 11 effects of early-life exercise but only three effects of early-life Western diet on adult traits, which suggests that the former may be a more effective target for interventions with human youth. Evidence concerning whether activity levels of children can be increased in particular areas without compensation in others is the subject of ongoing debate over a proposed homeostatic compensatory mechanism known as an "activity-stat" [50,51,172]. In any case, studies of declining physical activity in children suggest that sedentary behaviors begin displacing physical activity habits much earlier than is generally believed and that interventions should begin prior to initial school admission [52,173].

Our results may also be relevant to the current COVID-19 pandemic, wherein the lack of regular physical activity caused by lockdowns and other restrictions on mobility (and likely effects on diet) is becoming a widespread epidemiological issue [174]. In particular, children are poised to develop simultaneous lifestyle habits of physical inactivity and high consumption of calorie-dense foods in quarantine that may have lasting effects into adulthood, such as overweight and obesity [175]. (Of course, SARS-CoV-2 infection itself may be viewed as an early-life environmental factor with potentially long-lasting adverse effects.) Changes in lifestyle habits related to diet and exercise appear to differ by country. For example, Di Renzo et al. [176] found increased physical exercise and consumption of Mediterranean diet in Italy during the pandemic, whereas Sidor and Rzymski [177] found increased consumption of fast foods (which are usually high-fat, high-sugar) in Poland. In a survey of adults in Australia, 48.9% of respondents reported reduced daily physical activity as a result of pandemic confinement [178]. In another survey conducted on young people (aged 10 to 19 years) in a number of South American and European countries, respondents reported a significant increase in the intake of foods high in fat and sugar since the pandemic began [179]. Because the pandemic is fairly recent, long-term effects of these lifestyle changes are yet unknown; however, we predict that the COVID-19 pandemic will have long-lasting effects on physical activity and food preferences as today's affected children become adults. Given the acute maladies associated with physical inactivity and the virus itself [180,181], it seems especially important to implement interventions that may engender better lifestyle habits in children, who now experience the dietary and physical activity challenges that come with school closures and reduced access to recreational activities [175,182].

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2021.113389.

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