

## Original Article

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# Effects of early-life voluntary exercise and fructose on adult activity levels, body composition, aerobic capacity, and organ masses in mice bred for high voluntary wheel-running behavior

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## Abstract

Fructose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) is acutely obesogenic and is a risk factor for hypertension, cardiovascular disease, and nonalcoholic fatty liver disease. However, the possible long-lasting effects of early-life fructose consumption have not been studied. We tested for effects of early-life fructose and/or wheel access (voluntary exercise) in a line of selectively bred High Runner (HR) mice and a non-selected Control (C) line. Exposures began at weaning and continued for 3 weeks to sexual maturity, followed by a 23-week "washout" period (equivalent to ~17 human years). Fructose increased total caloric intake, body mass, and body fat during juvenile exposure, but had no effect on juvenile wheel running and no important lasting effects on adult physical activity or body weight/composition. Interestingly, adult maximal aerobic capacity (VO<sub>2</sub>max) was reduced in mice that had early-life fructose and wheel access. Consistent with previous studies, early-life exercise promoted adult wheel running. In a 3-way interaction, C mice that had early-life fructose and no wheel access gained body mass in response to 2 weeks of adult wheel access, while all other groups lost mass. Overall, we found some long-lasting positive effects of early-life exercise, but minimal effects of early-life fructose, regardless of the mouse line.

## Key findings

- Early-life exercise has numerous positive effects on adult traits.
- Early-life fructose consumption has minimal impacts on adult health.
- Exercise acutely protects against obesogenic effects of fructose in juvenile mice.

## Introduction

Compared with fat and other sugars, mammalian fructose metabolism involves specific intestinal transporters (GLUT5) and distinct inter-organ trafficking mechanisms. These aspects of fructose metabolism allow rapid energy assimilation and efficient energy storage, which are of particular benefit to animals that must accumulate energy prior to migration or hibernation.<sup>1</sup> Fructose metabolism may also benefit humans during times of food scarcity; however, excessive and/or continuous consumption of fructose can lead to insulin resistance and nonalcoholic fatty liver disease (NAFLD), especially in the absence of regular physical activity and the presence of consistent food availability.<sup>2,3</sup> As compared with other sugars and carbohydrates, fructose may present metabolic challenges because it bypasses a key enzymatic regulatory step in glycolysis (phosphofructokinase) and is rapidly metabolized in the liver, leading to the overproduction of pyruvate product and, consequently, an overproduction of lactate, glucose, and fatty acids. If allowed to continue in the long-term, unchecked fructolysis can potentially lead to such metabolic ailments as lacticemia, hyperlipidemia, and hyperuricemia,<sup>4</sup> especially in modern societies.

As fructose became available in the marketplace in the early 1970s, it immediately saw a meteoric rise to prominence among sweeteners in the United States, owing to its low cost in manufacturing, relative sweetness, usefulness in sweetened beverages, and improved shelf-life, among other properties.<sup>5–7</sup> At present, ~10% of caloric intake is derived from fructose in the United States, with children consuming the most fructose, primarily in the form of

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sugar-sweetened beverages (SSBs).<sup>6,8</sup> The overconsumption of fructose is of epidemiological interest for its contribution toward the development of obesity and other diseases, such as type 2 diabetes, NAFLD, certain cancers, and cardiovascular and kidney diseases.<sup>9</sup> Additionally, some research on laboratory mice suggests fructose may even reduce voluntary physical activity.<sup>10,11</sup> If true, then the overconsumption of fructose may represent a unique metabolic predicament in that it affects two lifestyle risk factors for obesity – physical inactivity and unhealthy eating behavior. Furthermore, “flavor learning” (which takes place during early infancy<sup>12</sup>) may play an important role in the development of obesogenic feeding patterns.<sup>13,14</sup> Taken together, this evidence suggests that early-life consumption of SSBs might have lasting effects on adult activity levels and food consumption habits, which in turn may lead to further dysregulation.

Early-life overconsumption of fructose or, more generally, of a Western diet (high in fat and sugar), does not occur in isolation from other risk factors for adult obesity and related diseases. Rather, in Western societies, children and adolescents often also experience a lack of physical activity, especially under recent COVID-19 pandemic conditions.<sup>15,16</sup> On the other hand, early-life exercise might counteract negative effects of fructose, as exercise during this period has been shown to have a number of positive effects on adult activity levels and caloric intake.<sup>17–19</sup>

Although some early-life studies in rodent models have demonstrated effects of high-fructose intake on adult memory,<sup>20,21</sup> the microbiome,<sup>22</sup> thermoregulation,<sup>23</sup> and the development of arterial hypertension,<sup>24</sup> no previous study has examined effects on adult physical activity and related traits. Moreover, the possibility that early-life exercise might serve as a countermeasure to adverse effects of fructose overconsumption has not been studied (though physical activity in general is known to modulate the health effects of fructose<sup>25</sup>).

Here, we used two lines from a unique mouse model, which includes four replicate High Runner (HR) mouse lines that have been selectively bred for high voluntary wheel-running on days 5 and 6 of a 6-day running period as young adults for more than 80 generations and compared it with one of the nonselected Control (C) mouse lines. HR lines run 2.5–3 times more revolutions per day when given access to wheels<sup>26,27</sup> and are more active in their home-cages without wheels.<sup>28</sup> The high levels of physical activity in HR mice might increase sensitivity to any adverse effects of early-life fructose supplementation, or possibly confer resistance.<sup>29–31</sup> HR lines show changes in other relevant traits as well, including reduced body mass and body fat, increased heart mass, increased maximal aerobic capacity, altered levels of circulating corticosterone, adiponectin, and leptin, and an altered brain reward system.<sup>17,30,32–36</sup>

Previously, the HR lines (but not C lines) have been shown to increase wheel running while given Western diet continuously from weaning through adulthood.<sup>27</sup> However, in a separate study, when administration of Western diet was restricted to the period from weaning to sexual maturity (3 weeks), followed by 8 weeks of “washout” period, it increased adult wheel running in both HR and C lines.<sup>17</sup> The discrepancy between these two studies is potentially attributable to seasonal variation in wheel running.<sup>26</sup> However, the source of dietary sugar in the Western diet mouse chow used in those studies (Harlan Teklad TD.88137) is sucrose (i.e., glucose + fructose) rather than only fructose.<sup>27</sup> Regular physical exercise, which increases production of brain-derived neurotrophic factor (BDNF) and affects the dopaminergic system,<sup>37,38</sup> may

therapeutically weaken sugar addiction. Exercise may also act as a competing reward in the brain<sup>36</sup> (and references therein).

Therefore, the purpose of the present study was to test for effects of fructose on physical activity levels in mice selectively bred for wheel-running behavior. Accordingly, we administered HR and C mice 30% weight/volume (w/v) fructose-water and/or access to exercise wheels during the 3-week early-life period between weaning and sexual maturity, similar to Cadney *et al.*<sup>17</sup> (see Fig. 1). After a 23-week “washout” period (i.e., a period of time when all animals were housed without wheels and with tap water) (equivalent to ~17 human years<sup>39</sup>), adult testing of wheel-running, cage activity, sucrose-preference, and maximal aerobic capacity began. Then, organ masses were measured in two separate cohorts: adults with and without 2-weeks of adult wheel access. We hypothesized that: 1) early-life exercise would affect adult traits, as previously demonstrated in both the HR and C mice<sup>17</sup>; 2) early-life fructose would suppress adult activity levels; and 3) early-life effects would interact with genetic background (i.e., effects would differ between of HR and C mice). Our hypotheses are related to the overarching idea that adult health is both affected by innate genetic variation and “programmable” by variation in environmental conditions.<sup>40–44</sup>

## Materials and methods

### Experimental mice

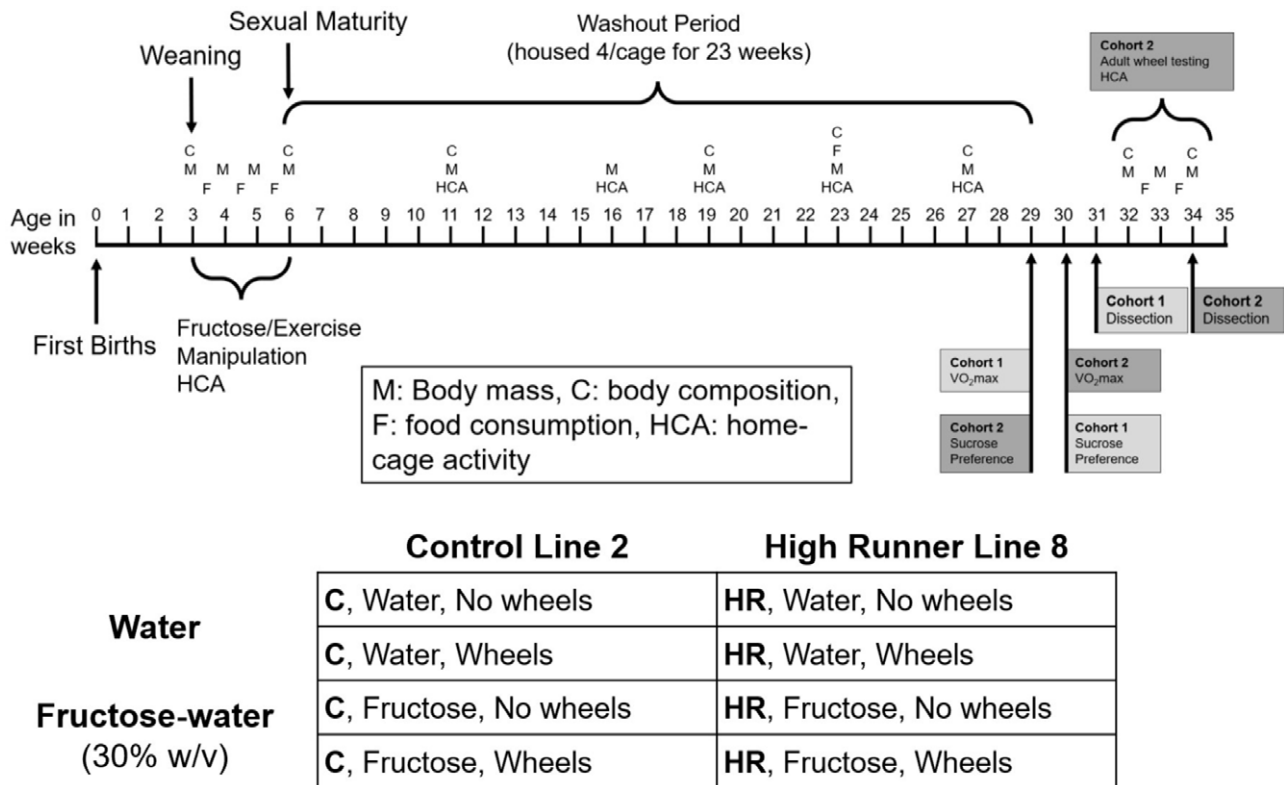
Starting in 1993, four replicate lines of house mice were bred in an ongoing selection experiment for high voluntary wheel running (HR lines), based on wheel revolutions on days five and six of a 6 day period of access to Wahman-type activity wheels (1.12-meter circumference) as young adults.<sup>45</sup> 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 lines were bred randomly as Control (C) lines alongside the four HR lines. A subset of mice ( $n = 104$ ) was sampled from generation 84, representing 34 families, for use in the current study. Mice from each family were distributed as evenly as possible between the treatment groups described below to avoid litter (dam) effects (see also *Statistical analysis*). For logistical reasons, we used female mice from only HR line 8 and C line 2 of the selection experiment (see Limitations section in Discussion).

### Early-life diet and exercise manipulation

In this experiment, 104 female mice were weaned individually into standard clear plastic cages ( $27 \times 17 \times 12.5$  cm) at 3 weeks of age and placed in one of four treatment groups for 3 weeks until sexual maturity at 6 weeks of age (see Fig. 1). Half of all mice were given 30% fructose-water w/v (commonly used in mouse models: e.g., Dotimas *et al.*,<sup>46</sup> Cho *et al.*,<sup>47</sup> and Tripathi *et al.*<sup>48</sup>) in standard cages, with half of the cages attached to activity wheels. Spontaneous physical activity (SPA) was measured as home cage activity using infrared sensors placed in home cages (see below). Body mass, food consumption, and body composition were measured during exposure (see Fig. 1 for a full account of these measurements). Photoperiod was 12:12, with lights on at 07:00 PST.

### Adult testing

Beginning at 6 weeks of age, all mice remained individually housed with standard chow,<sup>27</sup> *ad libitum* drinking water, and without



**Fig. 1.** Experimental timeline of events starting with the first births of generation 84 mice. Of these mice, 104 females (representing 36 families) were weaned and housed individually for the duration of the experiment. Early-life diet and exercise manipulation began immediately after weaning, lasted 3 weeks, and was followed by a 23-week washout period (equivalent to ~17 years for humans), during which mice were housed 4/cage without wheels and given a standard diet and regular drinking water (mice were individually housed during periodic washout measurements). At the end of washout, all mice were tested in two cohorts. Only Cohort 2 received wheel testing (2 weeks) so that two sets of dissected organ tissues could be collected – one with and one without having had recent adult exercise that may have caused training effects. The diagram shows the three-way experimental design (yielding four experimental groups) used to investigate the potential interactive effects of fructose in drinking water and access to exercise for 3 weeks during the juvenile period, from weaning to sexual maturity. Each cell represents  $n = 26$  female C and HR mice, for a total  $n = 104$ .

wheel access for an additional 23 weeks of washout. At 29 weeks of age, testing of  $VO_2$ max and sucrose preference began in two cohorts. Cohort 1 was dissected at week 31 without having received access to wheels for adult testing. During weeks 32–34, cohort 2 received wheel access for 2 weeks to measure voluntary exercise (VE). Adult SPA was again measured for the duration of wheel testing. Cohort 2 was dissected at week 34, immediately following wheel testing. Dissected tissue samples from cohorts 1 and 2 allowed for separate analyses of mice with and without adult access to exercise wheels.

**Home-cage activity**

During wheel testing and throughout the washout period, mice were housed in home cages fitted with a passive infrared sensors (Talon TL-Xpress-A; Crow Electronics, Fort Lee, New Jersey, USA), protected within wire mesh, as in previous studies.<sup>28,49</sup> 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, California, USA). A mean value between 0 and 1 was calculated for each minute over 23 hours. Analyses of SPA data used a measure of sensor sensitivity as a covariate.<sup>28,49</sup> During washout, mice were taken from their co-housed cages and housed individually so that SPA could be measured for 3 days.

**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) for scanning. Composition was analyzed throughout the experiment (see Fig. 1).

**Total caloric intake**

Food consumption was measured by the difference in food hopper weight each week during early-life treatment and adult wheel testing (only standard chow), taking care to note wasted or shredded food.<sup>50</sup> Weekly chow (solid food) consumption was converted from grams to caloric intake, taking the caloric content of standard chow into account (14.00 kJ/g<sup>27</sup>). Where mice were given fructose-water (15.40 kJ/g; USDA), those calories were added to obtain total caloric intake.

**Maximal aerobic capacity ( $VO_2$ max)**

The maximal rate of whole-organism oxygen consumption attained during graded exercise is considered the single best indicator of functional capacity relevant to sustained exercise and cardiorespiratory fitness,<sup>51</sup> but see Sadowska *et al.*<sup>52</sup> In turn, cardiorespiratory fitness is an independent predictor of health and mortality.<sup>53,54</sup>

VO<sub>2</sub>max was measured during forced exercise within a 900 mL enclosed mouse wheel ~15 cm in diameter.<sup>30,55</sup> Each mouse was run for approximately 4 minutes, with baseline values obtained before and after exercise. Duplicate trials were conducted, allowing a day of rest between each trial. Air was pumped into the enclosed metabolic chamber at a rate of 2,000 mL/min. using a mass-flow controller. The concentration of O<sub>2</sub> in dried, CO<sub>2</sub>-free excurrent air was measured by an oxygen analyzer (S-3A Applied Electrochemistry, Inc., Sunnyvale, CA). After instantaneous correction,<sup>56</sup> VO<sub>2</sub>max was taken as the highest minute of oxygen consumption during either trial, as calculated with LabHelper software (Warthog Systems, [www.warthog.ucr.edu](http://www.warthog.ucr.edu)).

We subjectively assessed quality during and tiredness after each trial. Trial quality<sup>30,57</sup> was scored between 1, being least cooperative (the mouse resisted moving in the direction of rotational motion), and 5, being most cooperative (the mouse consistently ran with the direction of rotational motion). Trial tiredness was determined by how quickly the mouse recovered from the trial, where a score of 1 indicates spontaneous locomotion within the chamber 1 second or less after the end of each trial and a score of 3 indicating movement after 5 or more seconds.

In analysis of VO<sub>2</sub>max, age and body mass were used as covariates. However, trial quality and tiredness were not significant predictors and were excluded from the model. Trial quality and tiredness were also analyzed as dependent variables. The identity of the researcher conducting the trial was used as a random effect in all analyses of VO<sub>2</sub>max and subjective scores.

### Preference for sucrose solution

At weeks 29 and 30, adult mice were presented with water bottles containing a sucrose-water solution (10.5% sucrose: Fisher Scientific Certified ACS Grade) and regular water. Fluid consumption, with due allowance for spillage and evaporation, was measured after mice had 24 hours (10:00–10:00 hours) of choice. We tested for sucrose preference, rather than fructose preference, to allow for better comparability with other rodent studies.

### Dissections

Animals were euthanized and organs were dissected and weighed to 0.0001 g (heart ventricles, triceps surae muscles, brains, liver, spleen, cecum, and subdermal and reproductive fat pads). Organs were preserved at –80°C.

### Statistical analysis

Data were analyzed as covariance 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. Line (selected line 8 vs. control line 2), early-life fluid type (fructose-water vs. water), and wheel access (exercise vs sedentary) exposures were fixed effects – except in analyses of traits measured prior to or during early-life exposure (e.g., weaning mass, juvenile running distance). Effects of line, fructose, and exercise, as well as their interactions, were tested. Dam ID, nested within line was used as a random effect to account for potential litter effects. Outliers were determined at ~3 standard errors from the mean and removed. Supplemental Material (SM1) presents full statistical analyses with significance levels, least squares means, and differences of least squares means for all traits. Supplemental Material (SM2) presents similar results of organ

masses with cohort as an additional main effect and its various interactions included.

Statistical significance was judged at  $p \leq 0.05$ . However, excluding the results of nuisance variables (such as age and wheel freeness), and body mass when used as a covariate, SM1 and SM2 include 878  $p$ -values for the main effects of line, fructose, wheel access, and their interactions. Of these 878  $p$ -values, 171 were nominally significant at  $p \leq 0.05$ . If all null hypotheses were in fact true, then nearly 44  $p$ -values ( $0.05 \times 878$ ) would be  $<0.05$  by chance alone. In addition, these tests include a substantial amount of nonindependence because the same individuals were measured for all traits, some traits were correlated (e.g., wheel running on successive days), and many tests were interrelated (e.g., body mass and fat mass). Therefore, to compensate for nonindependence in multiple related tests, we used the positive false discovery rate (pFDR) procedure as implemented in PROC MULTTEST in SAS version 9.4 (SAS, Cary, NC). Based on this procedure, an adjusted critical value of ~0.008 would be appropriate for controlling the positive false discovery rate at 5%. All  $p$ -values reported in the text and tables are raw values (i.e., not adjusted for multiple comparisons), so readers are cautioned to keep this in mind. In the text, we emphasized discussion of  $p$ -values  $p \leq 0.008$ . However, because the power to detect interactions is substantially lower for detecting main effects<sup>58</sup> we do discuss some of the interactions with  $p$ -values larger than 0.008.

## Results

### Juvenile wheel running

Fructose did not affect daily wheel-running distance during any week of juvenile wheel running, but as expected, HR mice ran more than C during both weeks 5 and 6 (Fig. 2A). Average daily wheel-running distance gradually diverged between HR and C lines (HR > C) during the 3 weeks after weaning, with the weekly HR/C ratio increasing from 1.27 to 1.91 to 2.45, respectively (Fig. 2;  $p = 0.2163$ ,  $p < 0.0001$ , and  $p < 0.0001$ , respectively). Average and maximum speed diverged similarly. Fructose did have some effects on running speed and/or duration. During the first week of early-life treatments, fructose reduced average and maximum wheel-running speeds in HR mice, but not C (Fig. 2C, D) – although the line  $\times$  fructose interaction was significant only for maximum speed, an examination of the differences of least-squares means shows a significant reduction in both average and maximum speeds among HR mice and no significant differences among the C lines. The effect is lost statistical significance into the second week of treatments and completely vanished into the third (see SM1). Fructose progressively increased wheel-running duration, with the effect becoming statistically significant during the third week (Fig. 2B).

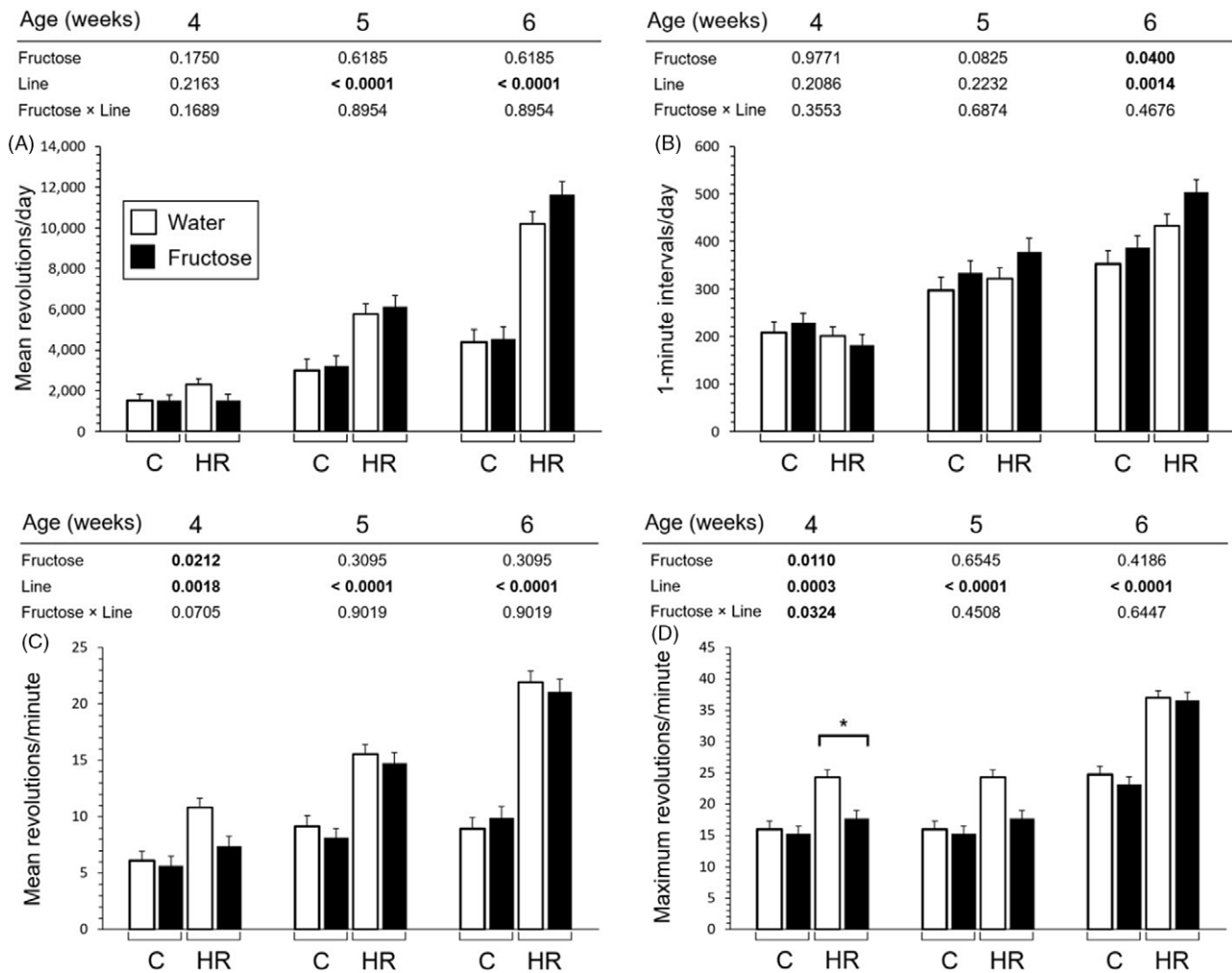
### Juvenile home-cage activity

Home-cage activity was recorded only for the third week of juvenile treatments. During the third week, fructose exposure increased activity in the home-cage ( $p = 0.0464$ ), wheel access decreased activity ( $p < 0.0001$ ), and HR mice were more active than C ( $p = 0.0009$ ), with no interactions of main effects (SM1).

### Adult wheel running

Early-life exposure to fructose did not significantly affect adult wheel running (Fig. 3). Early-life exposure to exercise generally





**Fig. 2.** Juvenile wheel running of female mice during 3 weeks of early-life treatment, shown as least-squares means, standard errors, and accompanying *p*-values from type 3 tests of fixed effects from SAS Procedure Mixed. These data are only from mice in the experimental exercise group (Table 1). Shown are mean values per day (circumference 1.12 m) for each week. White bars are mice from the early-life water treatment group and black bars the early-life fructose treatment group. Total sample size was ~52 female mice during each week. Asterisks highlight interaction effects, where the indicated comparison of least squares means was significant at *p* < 0.05.

increased adult wheel running; however, the effects on wheel-running distance and maximum speed were gone by the second week of testing (see SM1). HR mice ran more than C mice on all measures of wheel running across both weeks of testing (Fig. 3, SM1). Additionally, line and exercise had an interactive effect on running duration across both weeks of adult testing (line × exercise *p* = 0.0066 for week 1 and *p* = 0.0011 for week 2). An examination of differences of least squares means indicated early-life exercise significantly increased exercise duration in C mice, but not HR mice (Fig. 3B).

### Adult home-cage activity

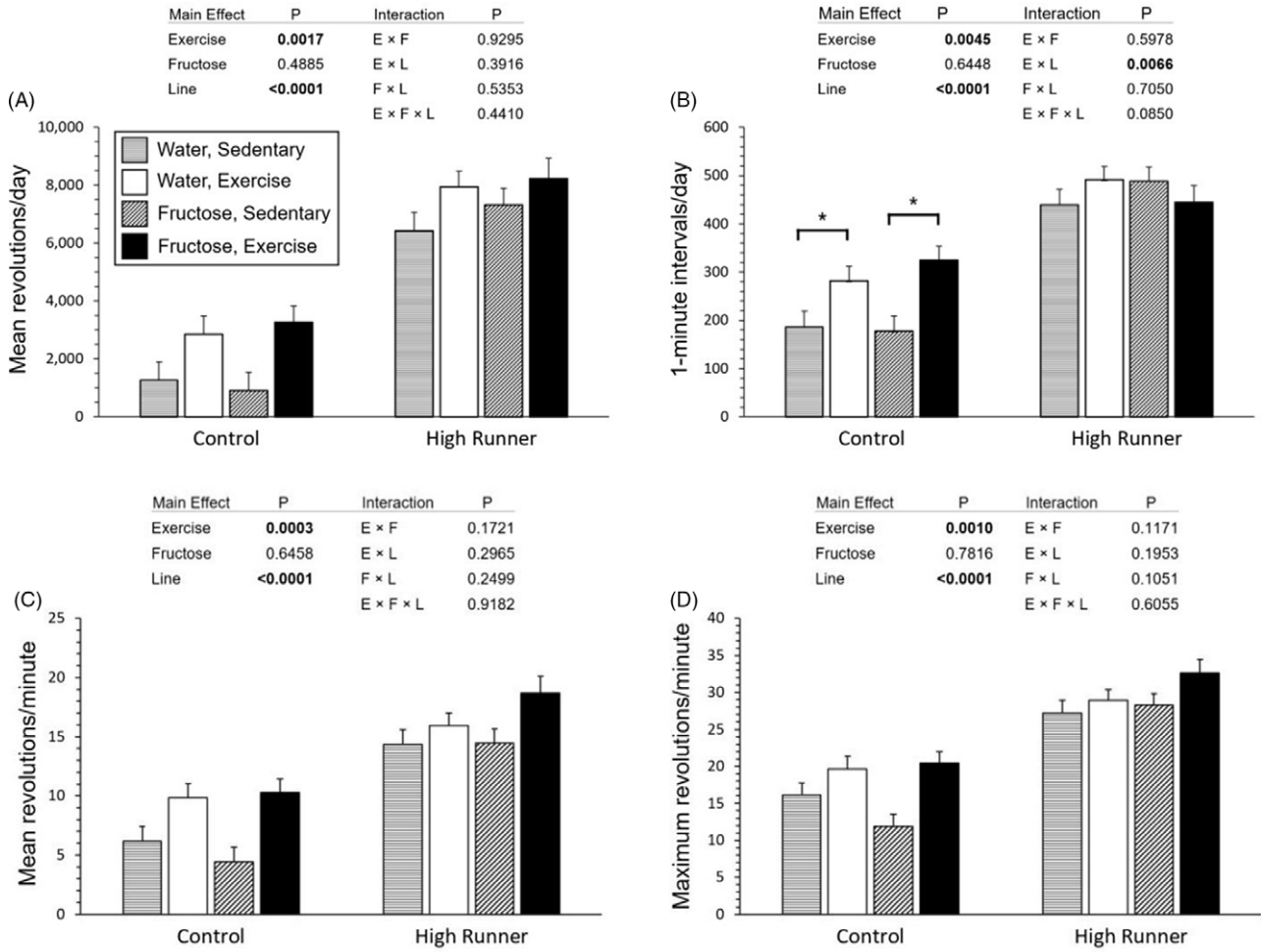
During the washout period, adult home-cage activity was significantly higher for HR than C mice during all weeks (all *p* < 0.0001; see SM1). Early-life exposure to fructose increased activity in the home-cage only during washout week 23 (*p* = 0.0098), and then again during the first week of adult wheel testing for cohort 2 (week 32 *p* = 0.0372). Early-life exposure to exercise decreased activity in the home-cage during the first week of adult wheel testing (week 32 *p* < 0.0001).

### Body mass

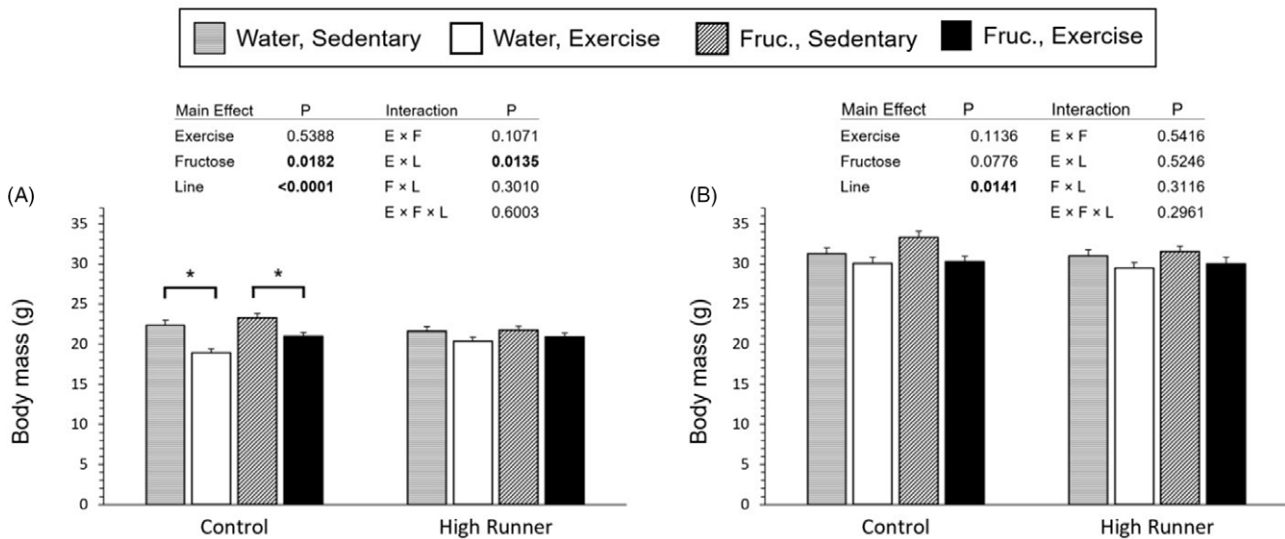
Fructose predictably increased body mass compared to the water group at the ends of weeks 4–6 (all *p* ≤ 0.0182) and into washout, where it increased body mass at weeks 16 (*p* = 0.0083) and 19 (*p* = 0.0419), but the effect was gone after week 19 (Fig. S1A, B). During the last week of early-life treatment (Fig. 4A), there was an interaction effect (exercise × line *p* = 0.0135). Inspection of the least squares means indicated that early-life exercise reduced body mass in C mice, but not HR mice. Early-life exposure to exercise temporarily decreased body mass during washout at week 19 (*p* = 0.0282), an effect that returned after 2 and 3 weeks of adult wheel testing (week 33 *p* = 0.0014, week 34 *p* = 0.0021). Overall, a comparison of body mass at weeks 6 and 23 (see Fig. 4) shows effects of growth and early-life experiences across washout; however, see SM3 for a more complete analysis of the time course.

### Fat mass

Repeated-measures ANOVA of fat mass indicated statistically significant interactions for exercise × fructose × line × trial (*p* = 0.0146), fructose × line × trial (*p* = 0.0429), and line × trial



**Fig. 3.** Adult wheel running during days 1–7 of a 2-week testing period. Values are least-squares means, standard errors, and accompanying *p*-values from type 3 tests of fixed effects from SAS Procedure Mixed. Asterisks highlight interaction effects, where the indicated comparison of least squares means was significant at *p* < 0.05. (A) Mean wheel revolutions per day, (B) duration of daily running, (C) mean revolutions per minute, (D) maximum revolutions per minute. Values for days 8–13 can be seen in SM1. Total *n* = 104 female mice.



**Fig. 4.** Body mass at weeks 6 and 23. Values are least-squares means, standard errors, and accompanying *p*-values from type 3 tests of fixed effects from SAS Procedure Mixed. Asterisks highlight interaction effects, where the indicated comparison of least squares means was significant at *p* < 0.05. (A) Body mass immediately after 3 weeks of early-life treatment (at week 6). (B) Body mass after 17 weeks of washout (at week 23).

**Table 1.** Fructose-induced increases in caloric intake

Wheel access	Line	Week 4	Week 5	Week 6
Sedentary	2 (C)	52.50	43.01	50.67
	8 (HR)	50.72	50.17	52.19
Exercise	2 (C)	35.24	25.98	77.89
	8 (HR)	58.97	77.29	90.87

During all 3 weeks of juvenile exposure, mice with fructose in the drinking water consumed more total calories than their counterparts without fructose. Values are differences in caloric intake for group with and without fructose (kJ/day).

( $p = 0.0038$ ) across the washout period (see SM1). Therefore, we examined each week separately.

Fructose did not affect fat mass as a main effect but was involved in three- and two-way interactions. Mice from the sedentary, fructose group had increased body fat compared to other groups after 3 weeks of early-life treatment (exercise  $\times$  fructose  $p = 0.0160$ ). Mice from the water, sedentary group had increased body fat in C and decreased body fat in HR (exercise  $\times$  fructose  $\times$  line  $p = 0.0075$ ) at week 32. Early-life exposure to exercise generally decreased fat mass. During juvenile exposure, the effect was never statistically significant, but it was during adult wheel testing at weeks 33 ( $p = 0.0215$ ) and 34 ( $p = 0.0280$ ). HR mice had less fat mass than C mice from weaning ( $p = 0.0212$ ) throughout the experiment (Fig. S1E). Additional results for fat and lean mass may be found in SM3.

### Caloric intake

Despite a decrease in the energy derived from chow (Fig. 5A), fructose increased total caloric intake (chow + fructose) in all groups (Fig. 5B). In other words, as shown in Table 1, mice with fructose in their drinking water did not fully compensate their energy intake. HR mice consistently consumed significantly more total calories than C mice across each week of juvenile treatment. During the second week, HR mice consumed even more total calories when also given fructose (fructose  $\times$  line  $p = 0.0120$ ).

After 17 weeks of washout, early-life exercise reduced food consumption in all groups ( $p < 0.0001$ ), and the three-way interaction was also significant (exercise  $\times$  fructose  $\times$  line  $p = 0.0302$ ).

During the first week of adult wheel testing, as would be expected, HR mice consumed more chow than did C mice ( $p < 0.0001$ ), mice with early-life wheel access consumed more chow ( $p = 0.0132$ ) in three of four groups, with a significant three-way interaction (exercise  $\times$  fructose  $\times$  line  $p = 0.0389$ ). During the second week of adult testing, only the effect of genetic line remained statistically significant (main effect of line  $p = 0.0013$ ; main effect of exercise  $p = 0.1554$ ; exercise  $\times$  fructose  $\times$  line  $p = 0.0747$ ).

### Maximal aerobic capacity

When cohorts were analyzed together (with age and body mass as covariates),  $VO_{2max}$  was affected by a two-way interaction: the combination of early-life wheel access and fructose reduced adult  $VO_{2max}$  (Fig. 6; exercise  $\times$  fructose  $p = 0.0144$ ). Trial quality did not differ across any group; however, trial tiredness was affected by line, with C mice being more tired after a trial than HR mice (see SM1;  $p < 0.0001$ ).

### Preference for sucrose-water

Preference for sucrose-water was not affected by line or by either early-life treatment, with no interactive effects (see SM1 for further statistical details).

### Organ masses

For mice that were not adult wheel-tested (cohort 1), we found few effects of early-life fructose exposure on organ masses, and little evidence for line differences (SM3). However, the liver was larger in HR than C mice ( $p = 0.0027$ ) and, as reported in several previous studies,<sup>17,59–61</sup> HR mice had larger hearts than C in all groups ( $p = 0.0035$ ). The fructose  $\times$  exercise interaction was also significant ( $p = 0.0250$ ), such that the combination of fructose and exercise treatment reduced ventricle mass (as it did for maximum aerobic capacity). In addition, early-life exposure to exercise decreased reproductive fat pad mass (Fig. S3H;  $p = 0.0205$ ).

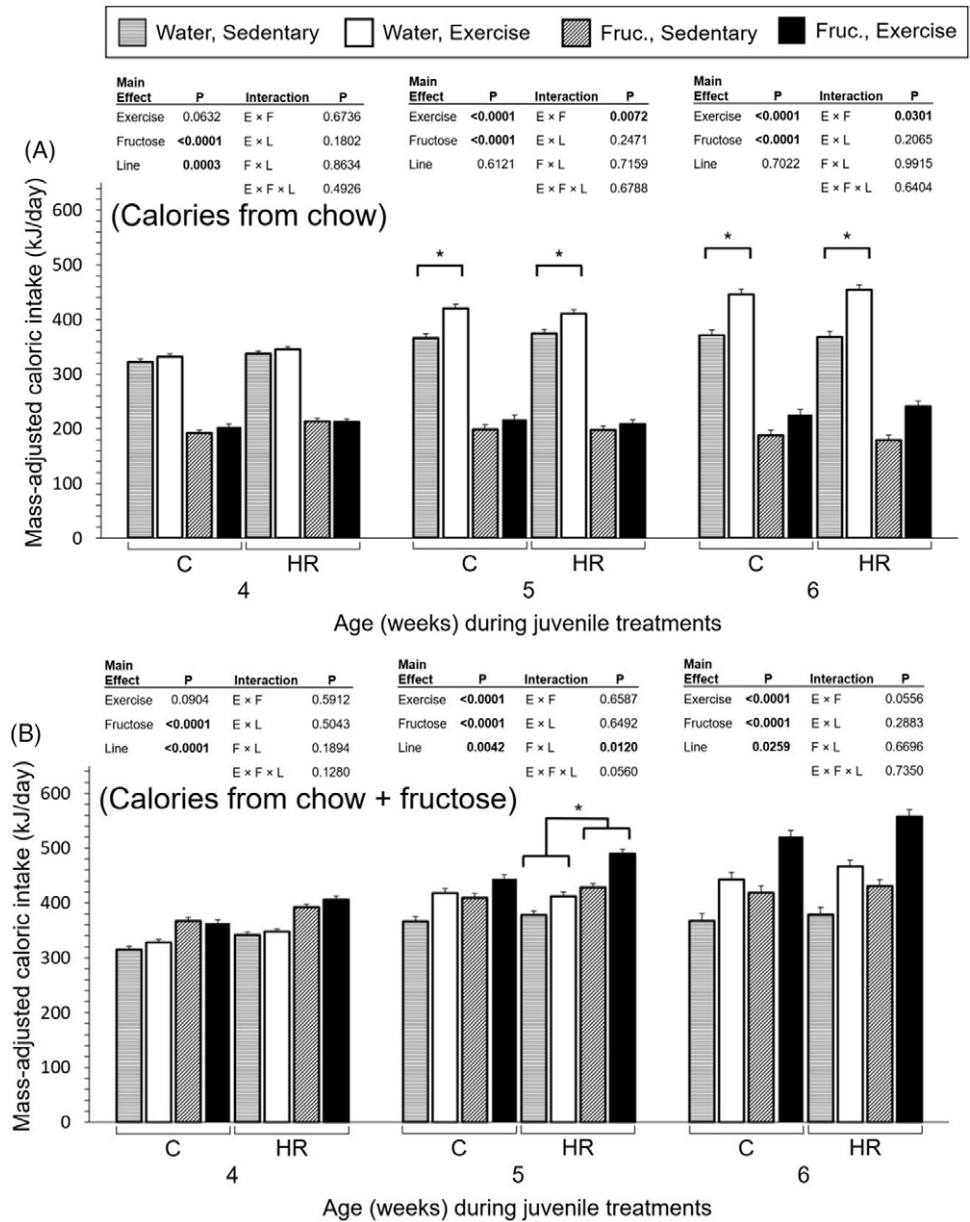
Combined analyses of cohorts 1 and 2 indicated several main and interactive effects. For example, with body mass as a covariate, heart ventricle mass was affected by line ( $p < 0.0001$ ), cohort ( $p < 0.0001$ ), and a line cohort interaction ( $p = 0.0176$ ); HR mice had larger hearts, wheel access increased heart mass, and the training effect was greater in HR mice. Full statistical analyses are reported in SM3.

### Discussion

In the present study, mice from a HR line selectively bred for voluntary wheel running and from a nonselected Control (C) line were administered fructose along lasting and/or access to exercise wheels during the 3-week period between weaning and sexual maturity. Numerous acute effects were detected during the treatment period, including obesogenic effects of fructose and fat-reducing effects of wheel access. When mice were tested as adults (after a 23-week washout period), early-life fructose had no detectable effect on daily wheel-running distance (or any of its components), but increased home-cage activity by a small amount (~4%). As predicted, some early-life effects on adult traits were interactive, including an early-life exercise effect that increased adult wheel-running duration in C (but not HR) mice during both weeks of adult wheel testing. In addition, we found some early-life effects and adult training effects (caused by 2 weeks of wheel access) on organ masses. Overall, we found that early-life exercise was responsible for the majority of effects on adult traits; fructose produced acute effects on activity levels; and HR and C mice were affected differentially by early-life treatments, in both the short- and long term. We found little evidence that fructose intake changed the response to exercise or that exercise changed the response to fructose differentially in the two lines.

### Differential effects of fructose on HR and C mice

Some fructose effects on HR and/or C mice were interactive, but the effects were mostly transitory. For instance, fructose reduced juvenile average and maximum wheel-running speeds (Fig. 2C, D, respectively), which is consistent with reports that fructose could suppress physical activity. An examination of the differences of least squares means shows the main effect is driven mostly by reductions in HR mice. The effect vanished by the third week of juvenile wheel running. At the same time, fructose briefly increased total caloric intake among HR mice (but not C mice) during the



**Fig. 5.** Weekly mass-adjusted juvenile caloric intake in response to juvenile fructose and/or exercise treatment. Asterisks highlight interaction effects, where the indicated comparison of least squares means was significant at  $p < 0.05$ . Weekly mass-adjusted caloric intake from chow only (A) and weekly total mass-adjusted caloric intake (chow + fructose) (B). Values are least squares means and standard errors from SAS Procedure Mixed. See SM1 for additional statistical details.

second week of juvenile wheel running (Fig. 5B). The nearly significant three-way interaction is worth noting, as HR mice on fructose and with wheel access consumed more total calories than any other group (exercise × fructose × line  $p = 0.0560$ ). Again, by the third week of wheel running, differential HR effects were gone. Differential effects on wheel running and caloric intake can be explained by elevated activity levels among HR mice (Fig. 2) driving greater total caloric intake, especially among HR mice that had fructose-water to drink.

Another interesting interactive effect involved  $VO_2$ max and relative heart size. Specifically, the exercise × fructose interaction was significant for both  $VO_2$ max and heart ventricle mass, such that the combination of early-life exercise and fructose treatment reduced both. This parallel effect suggests that the change in heart size could be causally related to the change in aerobic capacity. Arguing against this possibility, HR mice had larger ventricles than C mice, but not a significantly higher  $VO_2$ max.

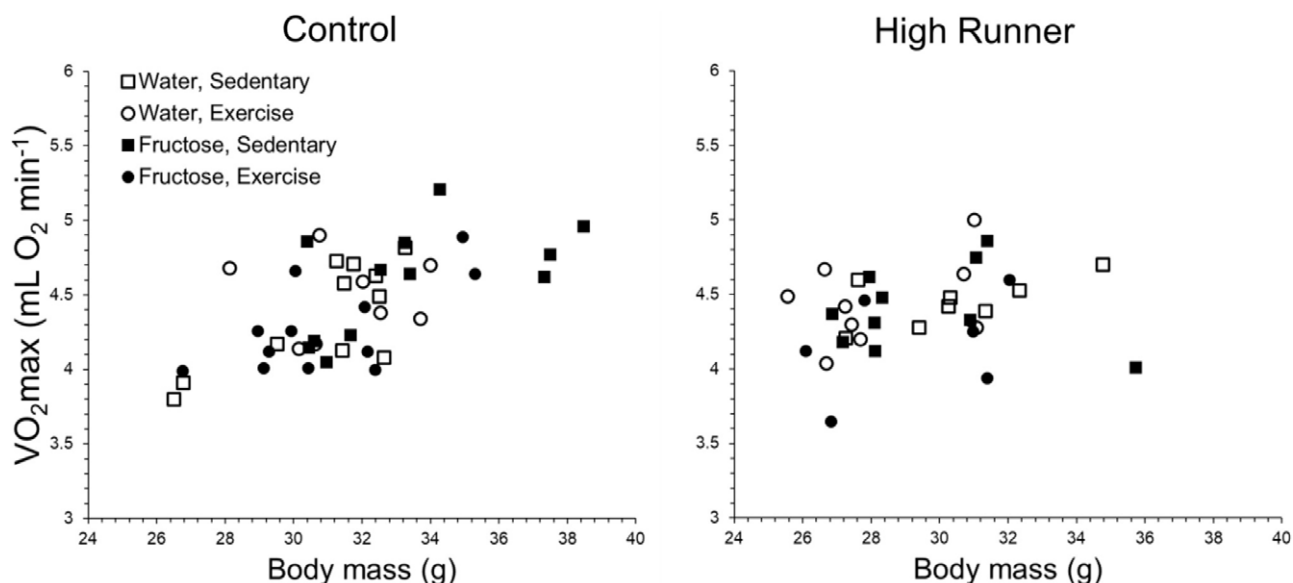
**Obesogenic effects of fructose**

Juvenile mice provided fructose in their water reduced the amount of chow eaten as compared with their counterpart experimental groups without fructose, but this compensation was not complete (Fig. 5). The excess caloric intake for mice with fructose ranged from ~26 to 91 kJ/day. The groups with fructose had greatly increased fat mass during the period of juvenile exposures (Fig. S1E–H). However, we found no lasting main effect of early-life fructose on adult body fat.

**Effects of early-life wheel access on adult physical activity**

The present and two previous studies all found long-lasting effects of early-life wheel access on adult running. However, Acosta *et al.*<sup>49</sup> and the present study found effects for both HR and C mice, whereas Cadney *et al.*<sup>17</sup> found effects only in C mice. This discrepancy may relate to measurements having occurred at different





**Fig. 6.** Maximum oxygen consumption ( $VO_2\max$ ) measured during forced exercise (see Methods). Analysis of  $VO_2\max$  was done with cohorts 1 and 2 combined (see SM1 for separate analyses). Total sample size ( $n = 77$ ) was reduced as a result of removing trials in which animals were uncooperative (see Methods). Body mass was a significant predictor of  $VO_2\max$  ( $p = 0.0005$ ), with no significant main effects.

times of year – wheel running shows strong seasonal variation, especially in the HR lines.<sup>26</sup>

#### Effects of fructose on physical activity

Rendeiro *et al.*<sup>10</sup> used two isocaloric diets containing either fructose or glucose at 18% of total metabolizable energy, where the diets replaced all sucrose and a fraction of cornstarch with either glucose or fructose. Mice received treatment diets for 11 weeks and then home-cage activity was measured via video tracking over 5 days. The fructose diet was gradually obesogenic over the course of the experiment, despite no statistical differences in food consumption (grams/body weight). The effect of fructose on body mass was attributed to reduced physical activity in the home cage, where the energetic expenditure of activity was estimated at 1.95 kcal/day for the fructose group and 2.44 kcal/day for the glucose group.<sup>10</sup>

We found mixed evidence regarding the acute effects of fructose on physical activity. During juvenile exposures, total home-cage activity (which we measured only during the third week) was significantly increased by fructose ( $p = 0.0464$ ) in the analysis of all mice (SM1), but the effect was not significant when we considered only the mice without wheels ( $p = 0.3740$ ). In none of the 3 weeks did we detect an effect on average daily running distance. During the first week of early-life exposure, fructose decreased average wheel-running speed in both HR and C mice ( $p = 0.0212$ ) and maximum speed only in HR mice (fructose  $\times$  line  $p = 0.0324$ ); however, fructose increased the duration of wheel running ( $p = 0.0400$ ) during the third week. Comparisons of our results with those of Rendeiro *et al.*<sup>10</sup> are not straightforward, in part because we did not attempt to impose equivalent caloric intake among groups.

Vellers *et al.*<sup>11</sup> fed mice a Western diet supplemented with fructose-water for 9–11 weeks after weaning. When compared to a control group, mice on the experimental diet consumed more calories per day, had greater body fat, and reduced wheel-running distance in both sexes.<sup>11</sup> Previously, we reported that Western diet had no effect on wheel running in C mice, although it dramatically

increased running in HR mice.<sup>17,27,29</sup> Therefore, we suspect that the reduction in physical activity reported by Vellers *et al.*<sup>11</sup> is attributable to either chronic overfeeding or a specific effect of fructose, rather than Western diet.

#### Possible protective effects of early-life exercise

Exercise is reported to curb dyslipidemia (abnormally high levels of circulating lipids) in a study on healthy human subjects fed a high-fructose diet.<sup>62</sup> More generally, adequate regular physical activity is known to prevent and help reverse obesity, type 2 diabetes, and other metabolic ailments.<sup>63–69</sup> Therefore, we predicted that early-life exercise might blunt any adverse effects of early-life fructose. Indeed, what might be interpreted as a “protective” exercise  $\times$  fructose interaction was observed for juvenile body fat mass. Specifically, with or without lean mass as a covariate, mice given fructose and with no opportunity for exercise had significantly greater body fat than other groups at the end of the 3-week exposure period. However, the effects of early-life fructose disappeared in subsequent weeks.

#### Unfamiliar early-life conditions as stressors

Although acutely obesogenic, fructose did not have a lasting effect on adult body mass (after the washout period). Previous studies have established that HR mice have evolved to be smaller and leaner than C mice.<sup>27,32,35</sup> We also found these differences for mice housed without either early-life wheels or fructose. Interestingly, however, when HR and C mice were exposed to either early-life fructose, wheel access, or both, differences in adult body fat were not apparent (see SM1). We speculate that these effects may reflect differential responses to early-life “stress,” with stress being caused generally by conditions that are unfamiliar in the evolutionary history of house mice since being brought into a laboratory setting. Specifically, early-life exercise and/or overnutrition may trigger thrifty fat storing<sup>70</sup> in HR mice, or adaptive fat loss in C mice.

In an early-life stress study using mice, Yam *et al.*<sup>71</sup> induced stress by limiting nesting and bedding material for 1 week after

birth. Plasma leptin levels and leptin mRNA expression in white adipose tissue were measured 9 days (short-term) and 180 days (long-term) after birth – both were significantly reduced by early-life stress. Then mice were fed a Western diet 6–14 weeks after birth, which resulted in an obese phenotype in mice that had experienced early-life stress.<sup>71</sup> If our early-life treatments similarly affected leptin homeostasis (Cadney *et al.*<sup>17</sup> found that early-life exercise increased adult leptin concentrations), then it is possible that HR lines, which evolved significantly lower circulating leptin levels and body fat than C lines,<sup>32</sup> might have responded to early stress differentially. Further studies of the altered behavioral and physiological responses to early-life “stress” in HR mice are needed.

### Effects on the response to adult wheel access

When we provided adult mice with exercise wheels for 2 weeks (~18 human months), nearly all groups lost body mass and fat mass. This general pattern has been reported previously for both sexes of HR and C mice given 6 days of wheel access,<sup>35</sup> as is used in the routine selective breeding protocol. Mice from all but one group (see below) lost body mass and fat mass, and early-life wheel access increased the amount of body mass and fat mass lost across 2 weeks of adult exercise (Fig. S2). Early-life exercise also decreased adult caloric intake in the first week of adult exercise testing. These effects could be secondary consequences of elevated adult wheel running due to early-life wheel access (Fig. 3).

One group stood out with respect to changes in adult body mass and composition: C mice from the early-life sedentary fructose group actually gained body mass in response to 2 weeks of adult exercise, attributable primarily to a relatively large increase in lean mass, accompanied by less of a drop in fat mass than seen in the other groups (Fig. S2). Notably, this group in particular represents the comparatively “unhealthy” combination of genetic and experimental factors: C mice (fatter and predisposed for less physical activity than HR mice); fed excessive amounts of simple carbohydrates as juveniles; without any access to exercise wheels. That this “unhealthy” group had an aberrant response to adult exercise after such a long washout period is startling and suggests permanent alterations to some aspects of their exercise physiology and metabolism may have occurred. Future studies would be required to determine the biochemical and molecular mechanisms that might underlie such hypothesized changes. We can, however, suggest that any such effects were not mediated by either  $\text{VO}_2\text{max}$  or wheel-running behavior, given that they were not affected by early-life fructose.

When analyzed as a 4-way model of exercise, fructose, line, and cohort, we tested for possible training effects of 2 weeks of adult wheel running. We observed a cohort  $\times$  line interaction for heart mass: HR mice that were wheel tested had greater ventricle mass than all other groups. The amount of wheel running over the 2 weeks was not significant when added as a covariate (SM2), suggesting that the HR mice have increased adaptive plasticity, rather than just following a “more pain, more gain” pattern.<sup>59,72–74</sup> We also observed a cohort  $\times$  line interaction for both reproductive and subdermal fat pads: C mice that were wheel tested had smaller fat pads, but that was not true for HR mice. Cecum mass showed two different 3-way interactions that were not simple to interpret. Additionally, adult exercise increased cecum mass for all groups, which may be an effect of increased food consumption caused by wheel running.

### Limitations of the present study

Numerous studies have targeted fructose and other simple carbohydrates (added to common foods) as likely obesogens in Western societies.<sup>4,75</sup> However, these effects may be driven simply by excess calories, so some researchers have used isocaloric diets when including fructose as a component.<sup>76,77</sup> In the present study, however, we gave mice *ad libitum* access to fructose-water, which means that any effects reported here may simply be the result of increased caloric intake (Fig. 5), rather than effects of fructose *per se*. We chose the present study design because we wanted to maximize the probability of observing early-life effects, which we believed would be most likely with an *ad libitum* diet. In any case, we detected no early-life effects of *ad libitum* fructose on adult activity levels.

Another limitation of the current study is that only female mice were included because males are likely to fight and sustain serious injury when co-housed for lengthy periods. Because many early-life effects may be sex specific<sup>60,61</sup> and many aspects of physiology and behavior are sexually dimorphic in both mice and humans,<sup>26,35,59,61,78</sup> (and references therein), future studies should include both sexes. For example, gestational exposure to BPA in humans has sex-specific effects on the length of pregnancy and birth weight.<sup>79</sup> In a previous study involving the HR mice, maternal exposure to WD had sex-specific effects on grand-offspring adult wheel running.<sup>60</sup>

Given the quantity of statistical tests involved in this study, corrections for multiple comparisons were made (see Methods). With an adjusted critical  $p$  of ~0.008, some reported results will not indicate statistical significance. However, the overall conclusions reported here do not change in light of more stringent critical values.

Finally, the current study used only one HR and one C line. As described above, the HR animal model includes four replicate HR lines and four replicate C lines. Previous work on these animals has documented line-specific effects for a variety of traits,<sup>26,50,55,60,78,80</sup> so future studies should ideally include representatives from all eight lines. We used only two lines to keep the necessary sample size within our logistical capacities (constrained by the number of running wheels, home-cage activity sensors, and personnel). Readers are cautioned that any line effects reported here may not be representative, although we hasten to add that the choice of these lines was made purely on the basis of litter availability at the time of sampling generation 84 mice.

### Concluding remarks

Fructose and glucose are metabolized differently – fructose is metabolized primarily in the liver and leads to *de novo* lipogenesis and elevated triglyceride synthesis. Compared to glucose, fructose metabolism has relatively few regulatory steps and does not trigger an insulin or leptin response upon uptake. Whether the unique metabolism of fructose has been a causative agent in the historic surge in the rate of obesity is controversial.<sup>9,81,82</sup> As compared with sucrose or other simple carbohydrates studied in comparable ways (e.g., other dietary components controlled, isocaloric intake), consumption of fructose, *per se*, has not been shown to have adverse effects on metabolic health in either human or rodent studies.<sup>83–85</sup> The present study expands upon these results by demonstrating no long-lasting adverse effects of early-life fructose consumption. It also underscores the importance of regular physical activity – both

early in life and during adulthood – in regulating body weight and adult activity levels.

**Supplementary materials.** For supplementary material for this article, please visit <https://doi.org/10.1017/S204017442200054X>

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**Conflicts of Interest.** The authors declare that they have no conflict of interest.

**Ethical Standards.** The authors assert that all procedures contributing to this work comply with the ethical standards of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and has been approved by the University of California, Riverside Institutional Animal Care and Use Committee (IACUC).

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