**Supplemental Material 3**

*Body mass*

At weaning, HR mice weighed less than C mice (*p = 0.0404*), and this pattern continued at the ends of weeks 5 (*p = 0.0199*), and 6 (*p = 0.0001*) (Fig. S1A, B). Throughout washout, HR mice continued to weigh less than C mice, but the effect was significant only during weeks 16 (*p = 0.0008*), 19 (*p = 0.0344*), and 27 (*p = 0.0141*). Similarly, the effect persisted through adult wheel testing. At the end of juvenile treatment (week 6), line and exercise interacted such that HR mice were smaller than C mice, except in the exercise group (line × exercise *p = 0.0135*). At the end of adult wheel testing (week 34), early-life exercise reduced body mass in C mice, but not HR (line × exercise *p = 0.0413*). The change in body mass when given two weeks of adult wheel access had a 2-way (fructose × line *p = 0.0146*) and 3-way (exercise × fructose × line *p = 0.0680*) interaction. All groups lost body mass (mainly due to loss of fat mass: see below), except for C mice given juvenile fructose and no exercise opportunity (Fig. S2).

*Lean mass*

Early-life exposure to fructose increased lean mass throughout the washout period during weeks 11 (*p = 0.0018*), 23 (*p = 0.0156*), and 27 (*p = 0.0374*) (Fig. S1C, D). Early-life exposure to exercise reduced lean mass throughout the washout period and the effect was only non-significant at week 23 (*p = 0.0667*). After two weeks of adult wheel testing, early-life exposure to exercise decreased lean mass (week 33 *p = 0.0035*, week 34 *p = 0.0073*). Wheel access decreased lean mass in C mice but increased it in HR (line × exercise *p = 0.0086*). During washout, early-life exercise decreased lean mass in C mice, but not HR mice (week 19 line × exercise *p = 0.0319*). After the washout period and immediately prior to wheel testing, HR mice had less lean mass than C mice (week 32 *p = 0.0380*) and the effect persisted through adult wheel testing. After two weeks of adult wheel testing, early-life exercise decreased lean mass in C mice, but not HR mice (week 34 line × exercise *p = 0.0177*). When considering the change in lean mass across the 2 weeks of adult exercise, C mice gained lean mass (Fig. S2 and SM1: delta *p = 0.0010*), while HR mice remained unchanged.

*Fat mass*

In analyses with lean mass as a covariate (Fig. S1G, H), mice from the sedentary, fructose group had increased body fat compared to other groups after 3 weeks of early-life treatment (exercise × fructose *p = 0.0254*). HR mice from the fructose group, but not the water group had reduced body (fructose × line *p = 0.0280*) at week 11. Mice from the water, sedentary group had increased body fat in C and decreased body fat in HR (exercise × fructose × line *p = 0.0320*) at week 32. Early-life exposure to exercise generally decreased fat mass. The effect was significant only at the end of juvenile treatment (p < 0.0001), and at weeks 19 (*p = 0.0357*) and 32 (*p = 0.0227*).

HR mice generally had less fat mass than C mice throughout the experiment, but the effect was not significant during early-life treatment and at weeks 23 and 32 (Fig. S1G, H). All groups lost fat mass across 2 weeks of adult exercise (without lean mass as a covariate), and the reduction was significant in every group except HR mice on with early-life exercise and regular drinking water (Fig. S2).

*Organ masses for cohort 1*

With log body mass as a covariate, early-life exposure to exercise increased heart mass in the water group and decreased it in the fructose group (Fig. S3A; exercise × fructose *p = 0.0250*) for both HR and C mice. In addition, as reported in several previous studies 17,47–49, HR mice had larger hearts than C in all groups (*p = 0.0035*). Liver mass was not affected by early-life treatments but was larger in HR than C mice (Fig. S3D; *p = 0.0027*). Early-life exposure to exercise decreased reproductive fat pad mass (Fig. S3H; *p = 0.0205*). Early-life exercise increased brain mass in C but not HR mice (exercise × line *p = 0.0702*), and early-life fructose reduced brain mass in C but not HR mice (fructose × line *p = 0.0563*) (Fig. S3C).

*Organ masses for both cohorts combined*

Organ masses were analyzed in four-way analyses, adding cohort as a main effect to test for potential training effects caused by two weeks of wheel access. In each case, wheel-running distance was never a significant predictor of organ mass when used as a covariate, so it was removed from the final models (see SM2 for results of four-way ANOVAs with and without wheel running distance as a covariate).

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. Cohort and line interactively affected both subdermal (*p = 0.0018*) and reproductive (*p = 0.0443*) fat pads: in both cases, adult wheel access significantly reduced fat pad mass in C mice but did not significantly affect it in HR mice. Liver mass was increased by adult wheel access (*p < 0.0001*), larger in HR mice (*p = 0.0005*), and also affected by an exercise × fructose interaction (*p = 0.0490*). Triceps surae muscle mass was larger in HR mice (*p = 0.0496*) and was also affected by an exercise × fructose (*p = 0.0402*) and a cohort × fructose × line interaction (*p = 0.0236*). Adult wheel access had a consistent effect of increasing cecum mass (*p < 0.0001*), and two 3-way interactions were also statistically significant (see SM2).

**Figures**

Figure S1. Total body mass, lean mass, and fat mass. Values are least-squares means and standard errors from SAS Procedure Mixed. Note that body mass and composition were also measured at weaning (week 3) but are not shown here (see Results); HR mice at weaning weighed 10.3% less than C mice (p = 0.0404). Fat mass was analyzed with and without lean mass as a covariate. See SM1 for additional statistical details.

Figure S2. Change in body mass across two weeks of adult wheel access. Measurements were taken immediately before and after wheel testing (see Fig. 1). Values are least squares means from SAS Procedure Mixed. See SM1 for statistical details. \*Fat mass with lean mass as a covariate.

Figure S3. Body-mass adjusted heart ventricles, triceps surae, brain, liver, spleen, and cecum, and fat pad masses for cohort 1. 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. All traits were analyzed with body mass as a covariate (and with log body mass when the dependent variable was log-transformed [A, F]). See SM1 for additional statistical details.