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Mice selectively bred for high voluntary wheel-running behavior conserve more fat despite increased exercise

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Abstract

Physical activity is an important component of energy expenditure, and acute changes in activity can lead to energy imbalances that affect body composition, even under *ad libitum* food availability. One example of acute increases in physical activity is four replicate, selectively-bred High Runner (HR) lines of mice that voluntarily run ~3-fold more wheel revolutions per day over 6-day trials and are leaner, as compared with four non-selected control (C) lines. We expected that voluntary exercise would increase food consumption, build lean mass, and reduce fat mass, but that these effects would likely differ between HR and C lines or between the sexes. We compared wheel running, cage activity, food consumption, and body composition between HR and C lines for young adults of both sexes, and examined interrelationships of those traits across 6 days of wheel access. Before wheel testing, HR mice weighed less than C, primarily due to reduced lean mass, and females were lighter than males, entirely due to lower lean mass. Over 6 days of wheel access, all groups tended to gain small amounts of lean mass, but lose fat mass. HR mice lost less fat than C mice, in spite of much higher activity levels, resulting in convergence to a fat mass of ~1.7 g for all 4 groups. HR mice consumed more food than C mice (with body mass as a covariate), even accounting for their higher activity levels. No significant sex-by-linetype interactions were observed for any of the foregoing traits. Structural equation models showed that the four sex-by-linetype groups differed considerably in the complex phenotypic architecture of these traits. Interrelationships among traits differed by genetic background and sex, lending support to the idea that recommendations regarding weight management, diet, and exercise may need to be tailored to the individual level.

Keywords: body composition, fat, selection limit, spontaneous physical activity, voluntary exercise, wheel running

1. Introduction

Imbalances between energy intake and expenditure cause changes in body mass and composition that can be mediated by body size, sex, and genetic background (Pomp et al. 2008; McAllister et al. 2009; Kelly et al. 2011). One important cause of energy expenditure is physical activity, the major components of which are voluntary exercise (VE) and spontaneous physical activity (SPA) (Garland et al. 2011b; Thompson et al. 2012; Teske et al. 2014), although the definitions of VE and SPA are not always clear (review in Garland et al. 2011b). In humans, VE is generally self-evident and SPA is generally considered as all other physical activity that is not VE, including fidgeting and pacing (although “gray areas” exist, e.g., physical education classes in primary school). In rodents, VE is recorded by wheel running (Sherwin 1998) and SPA is recorded by home cage activity (Garland et al. 2011b). The relative importance of VE and SPA as sources of energy expenditure varies among species and with environmental conditions, and also depending on whether variation in either type of activity is caused mainly by variation in frequency, duration or average intensity (e.g., Koteja et al. 1999; Copes et al. 2015).

When the level of VE or SPA increases, animals may compensate by reducing energy expenditure related to the other component or during other aspects of the daily lifecycle; alternatively or in addition, they may increase food consumption (Westerterp and Plasqui 2004; King et al. 2008; Garland et al. 2011b). Such adjustments may or may not lead to stability in body mass and composition, depending on how long the altered physical activity occurs and the availability of additional food, as well as the sophistication of the organism's homeostatic mechanisms, such as appetite (e.g., see Blundell and King 1998; Piersma and Van Gils 2011). In general, animals that have evolved with a history of short-term changes in energy demand, as through temporarily increased levels of physical activity, would be expected to cope with those changes better than animals that are not adapted to such conditions. We tested this general proposition by comparison of lines of mice that vary genetically in levels of physical activity.

Specifically, we compared four replicate High Runner (HR) lines of mice selectively bred for increased wheel running during days 5 and 6 of a 6-day period of wheel access with four non-selected Control lines (Swallow et al. 1998). Mice from HR lines run ~3 times more distance per day than C mice over the 6-day period of wheel access (e.g., Belter et al. 2004; Garland et al. 2011a; Careau et al. 2013) and offer a unique model for studying the effects of acute increases in physical activity on (changes in) food consumption and body composition. Despite continued selection for increased levels of VE, all of the HR lines have been at a selection limit since generation 17-25, depending on line and sex (Careau et al. 2013). In principle, these limits could be related to an inability to maintain energy balance and body composition during the 6-day trial. Alternatively, the HR mice may have evolved mechanisms to compensate for the dramatically increased VE.

In addition to much higher VE, several other comparisons of HR and C lines suggest differences in their ability to regulate body mass or composition (Garland et al. 2011b, 2016; Wallace and Garland 2016). For example, HR mice are more active in home-cages when wheels are not provided (Malisch et al. 2009; Copes et al. 2015), eat more as adults even when housed without wheels (Swallow et al. 2001; Copes et al. 2015), are smaller in total body mass (Koteja et al. 1999a), with the difference more pronounced in males than females (Swallow et al. 1999; Garland et al. 2011a)), have reduced body fat (Swallow et al. 2001; Nehrenberg et al. 2009), reduced circulating leptin levels (Girard et al. 2007), and increased adiponectin levels (Vaanholt et al. 2007). Moreover, the amount of wheel running does not reach a plateau within six days in either HR or C mice (e.g., Swallow et al. 2001; Acosta et al. 2017), and neither does the amount

of cage activity, a measure of SPA (Acosta et al. 2017), or body mass (Swallow et al. 2001; Bronikowski et al. 2006). Thus, energy balance and body composition are likely still in flux when breeders are chosen each generation.

The purpose of the present study was to characterize the effect of sex and genetic background on initial body composition and on changes that occur during 6 days of voluntary exercise. Furthermore, within each of the four groups (C male, C female, HR male, HR female), we used structural equations to model the relative importance of various paths in the complex network of activity and body composition phenotypes at the level of individual variation (cf. King et al. 2008). Figure 5A presents a path diagram outlining expected relations among the measured traits, ignoring the possibility of sex-specific effects. In general, we expected that all four measures of physical activity (intensity and duration of VE and SPA) would be positive predictors of both food consumption (Copes et al. 2015) and fat loss. We also expected that VE would be associated with changes in lean mass, but the direction of the association is difficult to predict because strength training tends to increase muscle mass, whereas aerobic exercise can reduce it, so speed vs. duration of VE might have different effects. [Note that wheel running involves some degree of climbing-like locomotor behavior in large wheels as used here, which might tend to increase muscle mass, though perhaps in a genotype-dependent manner (e.g., see Lionikas and Blizard 2008).] We did not expect the components of SPA to affect changes in lean mass.

2. Methods

2.1. Mouse model

Creation and maintenance of the four replicate High Runner (HR) and Control (C) lines is described elsewhere (Swallow et al. 1998; Careau et al. 2013). Here, we used 348 mice from generation 77. Mice were weaned at 3 weeks of age and housed in standard cages of 4 mice by sex, with *ad libitum* food (Teklad Rodent Diet W-8604) and water, at 20-24 degrees Celsius with 12:12 light-dark cycles.

As young adults (age 46-70 days), mice were placed individually in clean cages with access to wheels for 6 days, as in testing for the routine selection protocol (1.12 m circumference: (see Fig. S1 in Kelly et al. 2017)). We assigned individuals to wheels balancing by sex and linetype. Wheel running was recorded with an automated counting system in 1-minute increments for each day. From this we obtained daily running distance (revolutions per day), duration (minutes per day with any activity), mean speed (revolutions per minute), and maximum speed (maximum number of revolutions in any 1-minute interval). Mice were similarly monitored for activity in the home-cage by passive infrared motion-detection sensors (Copes et al. 2015). Software recorded '1' (movement detected) or '0' (no movement detected) 3 times per second from the sensor and saved the mean value (between 0 and 1) every minute. From these data we obtained daily activity levels (arbitrary activity units), duration, mean intensity (activity units per minute), and maximum intensity (maximum activity units in any 1-minute interval). We analyzed wheel running and home-cage activity for the last two days of the 6-day trial (mean of days 5 and 6) because those are used in the selection protocol (Swallow et al. 1998).

We weighed mice and food hoppers (± 0.01 g) before and after wheel access, noting obvious signs of food wasting or shredding (Koteja et al. 2003). We used non-invasive, quantitative magnetic resonance to analyze body composition (EchoMRI-100, Echo Medical Systems, Houston, TX), independently determining lean and fat masses of each animal.

2.2. Conventional statistical analyses

Among-group differences were analyzed using covariance models with Type III tests of fixed effects in the Mixed Procedure in SAS 9.4M4 (SAS Institute, Cary, NC, USA). Sex, linetype (HR or C), and their interaction were included in the model as fixed effects. Random effects in the model were replicate lines nested within linetype, family identity nested within line and linetype, and sex-by-line interaction effects nested within linetype.

Total, lean, and fat masses were analyzed separately for before and after wheel access, and change in mass was calculated as mass after wheel access minus mass before wheel access. Analyses of masses included age and age-squared as covariates because mice were tested over a span of 4 weeks, which resulted in a curvilinear relationship. We obtained age-squared by standardizing age to have mean = 0 and standard deviation = 1 and then squaring those standardized values. Change in mass was also analyzed by repeated measures, but some models did not converge (Supplemental Table S1). Analyses of fat and lean percent are also available as supplemental material (Supplemental Table S2 and Fig. S2).

Analyses of food consumption used initial body mass as a covariate. We also used a model with covariates of activity levels (both intensity and duration of wheel running and home-cage activity).

Wheel running and component traits (duration, mean and maximum speed) were analyzed with age and wheel freeness as covariates. Rotational freeness was measured for each wheel by accelerating it to a constant speed for 5 rotations and counting revolutions until the wheel stopped on its own. Home-cage activity and component traits were analyzed similarly, but to obtain normality of residuals, total home-cage activity, duration, and mean intensity were log₁₀-transformed and maximum intensity was raised to the 2.5th power. We used covariates of age and infrared sensor sensitivity, which was calibrated by using a heating stick swung in the home-cage for 5 seconds and recording the activity reported by each sensor. Sensor sensitivity and wheel freeness were each square-rooted to obtain a normal spread of values and the mean of measurements taken before and after wheel access (with two measures per time) was used as a covariate.

2.3. Structural equation modeling analyses

To determine the complex phenotypic architecture of activity and body composition with each group, we analyzed our data using structural equation modeling in Onyx version 1.0-937 (von Oertzen et al. 2015). The variables tested were wheel-running speed and duration, home-cage activity intensity and duration, initial body mass, food consumption, change in fat mass and lean mass, and nuisance variable of age, age-squared, square-rooted wheel freeness, and square-rooted sensor sensitivity. We ran the same model separately for the four sex-and-linetype groups: female C, female HR, male C, and male HR. To account for known differences between the replicate lines (Garland et al. 2011a), we centered every dependent variable to have the same mean among the 4 replicate lines within sex-and-linetype groups. In the model, each variable was z-transformed, every variable had a variance fixed to 1.0, and every exogenous variable pair had covariances. All paths except variances were unfixed (freed parameters). Within each group, we used the parameter estimate and standard error for each path to obtain 95% confidence intervals (estimate $\pm$ 2 x SE) and significance was determined by the confidence interval being bound away from zero.

3. Results

3.1. Body, lean, and fat mass

To study the effects of physical activity on energy balance, we measured total body, lean, and fat masses before and after 6 days of wheel testing.

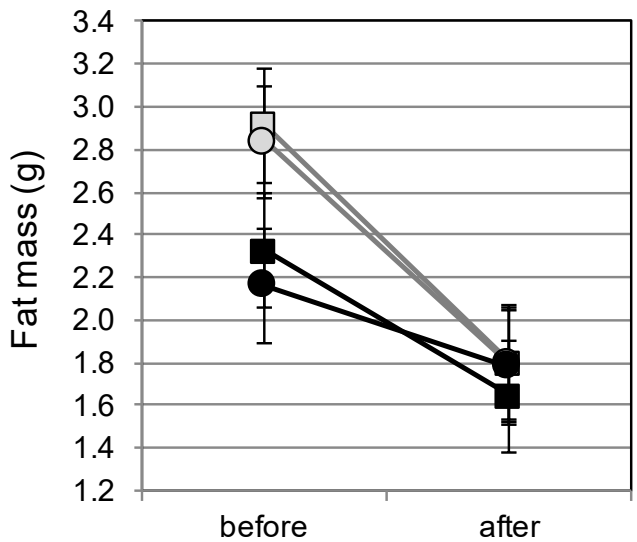
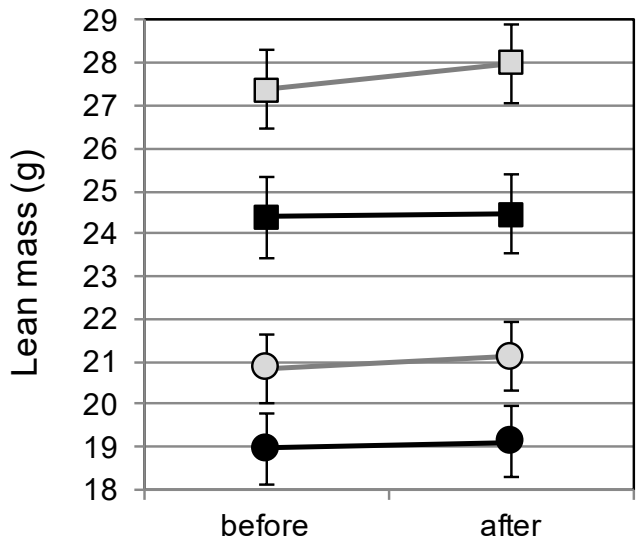
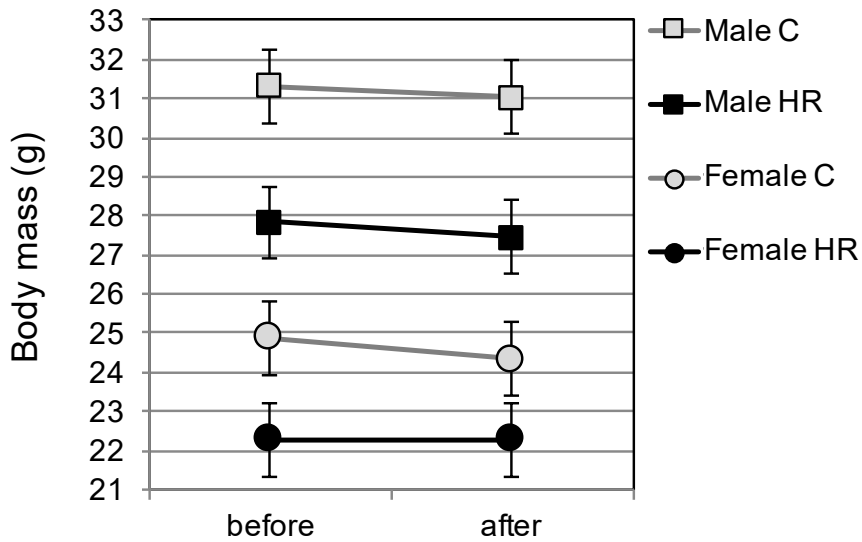
Before 6 days of wheel testing, body mass was significantly lower in HR mice ($p = 0.0489$, Table 1). This reduction was due mostly to reduced lean mass ($p = 0.0631$) as opposed to reduced fat mass ($p = 0.1185$, Table 1 and Fig. 1). Females also had significantly reduced body mass ($p < 0.0001$), which was entirely due to lower lean mass ($p < 0.0001$) and not fat mass ($p = 0.3234$, Table 1 and Fig. 1). Analyzed as percent body mass, lean mass was significantly lower ($p = 0.0041$) and fat mass was significantly higher ($p = 0.0007$) in females compared with males (Table 1 and Fig. 2).

All groups lost body mass after 6 days of wheel access ($p = 0.0342$) due to a significant loss in fat mass ($p < 0.0001$) and despite a tendency for increased lean mass (Table 1 and Fig. 1). The loss in body mass and the gain in lean mass were not significantly affected by sex, linetype, or their interaction ($p > 0.05$, Table 1). Wheel minutes, speed, and home-cage minutes were significant predictors of total body mass change ($p < 0.05$, Table 1), but using them as covariates did not change the main effects of sex and linetype.

HR lost significantly less fat mass than C mice ($p = 0.0133$, Table 1). After accounting for activity levels, the effect of linetype was not significant ($p = 0.2916$), but females tended to lose less fat ($p = 0.0518$, Table 1). Higher wheel-running duration resulted in greater fat loss ($p < 0.0001$) while higher running speed and minutes spent in home-cage activity resulted in decreased fat loss ($p < 0.0001$ and $p = 0.0002$, Table 1; see section 3.4. below for more detailed explanation of these effects).

Table 1. Significance levels (P values) from statistical analyses of body mass, body composition, food consumption, and activity levels during 6 days of wheel access. "Change" was calculated as after - before 6 days of wheel access. Main effects were sex (female or male), linetype (C or HR), and their interactions ("Sex x C vs HR"). Covariates used were age and age-squared (first standardized and then squared) [not shown]. Food consumption included an additional covariate of body mass. For analyses of change in masses and food consumption, additional covariates indicating activity metrics were used. For activity levels, covariates of wheel freeness or sensor sensitivity were used (results not shown). Home-cage total activity, duration, and mean intensity were log₁₀ transformed and maximum intensity was square-rooted prior to analyses to obtain normality of residuals. All statistically significant P values (<0.05) are in bold and signs following the value indicate direction of effect. Note, as all mice lost fat, the + sign after the linetype effect (C vs HR) means that HR lines lost less fat mass.

Trait	N	Sex	C vs HR	Sex x C vs HR	Wheel minutes	Wheel speed	Home-cage minutes	Home-cage intensity	Mass
Total mass									
Before wheel access	333	<0.0001+	0.0489	- 0.1587					
After wheel access	334	<0.0001+	0.0585	- 0.1191					
Change	321	0.8304	0.3174	0.2134					
Change with activity	321	0.5402	0.8687	0.4028	<0.0001-	0.0005+	0.0009+	0.8732	
Lean mass									
Before wheel access	333	<0.0001+	0.0631	- 0.1763					
After wheel access	333	<0.0001+	0.0507	- 0.1125					
Change	320	0.5397	0.2146	0.2816					
Change with activity	320	0.5620	0.1577	0.3584	0.0052-	0.2098	0.0473+	0.9948	
Fat mass									
Before wheel access	333	0.3234	0.1185	- 0.9132					
After wheel access	333	0.3455	0.7954	0.3410					
Change	320	0.1412	0.0133+	0.3428					
Change with activity	320	0.0518	- 0.2916	0.8877	<0.0001-	<0.0001+	0.0002+	0.3545	
Food consumption									
Before wheel access	312	0.1493	<0.0001+	0.3836					<0.0001+
With activity	306	0.8089	0.0031+	0.3946	0.0469+	<0.0001+	0.0033+	0.9975	<0.0001+
Total wheel running									
Duration	341	0.0061	-<0.0001+	0.5188					
Mean speed	338	0.0005	- 0.0480+	0.0287					
Maximum speed	340	0.0819	-<0.0001+	0.5339					
Total home-cage activity									
Duration	337	0.1155	<0.0001+	0.7363					
Mean intensity	343	0.5049	0.2651	0.7191					
Maximum intensity	346	0.0013	- 0.1889	0.3753					
	344	0.0321+	0.6639	0.1587					
	346	0.2222	0.5421	0.6252					



6 days of wheel access

Fig. 1. Total, lean, and fat mass measured for each mouse before and after 6 days of wheel access. Males had higher total and lean mass before and after the 6 days of wheel access. On average, mice lost body mass, gained lean mass, and lost fat mass. HR mice lost less fat, and the four groups converged to a fat mass of ~1.7 g after 6 days of wheel access. Values are least-squares means $\pm$ standard errors from analyses of covariance in SAS Procedure Mixed. Corresponding *P* values are in Table 1. Analyses included covariates of age and age-squared. Each point represents ~80 mice.

3.2. Food consumption

Adjusting for initial body mass before wheel access, HR mice consumed significantly more food than C mice ($p < 0.0001$), with no effect of sex. Running speed, duration, and home-cage activity duration were significant positive predictors of food consumption, but adding them as covariates did not change the main effect of linetype ($p = 0.0031$) or sex (Table 1 and Fig. 2).

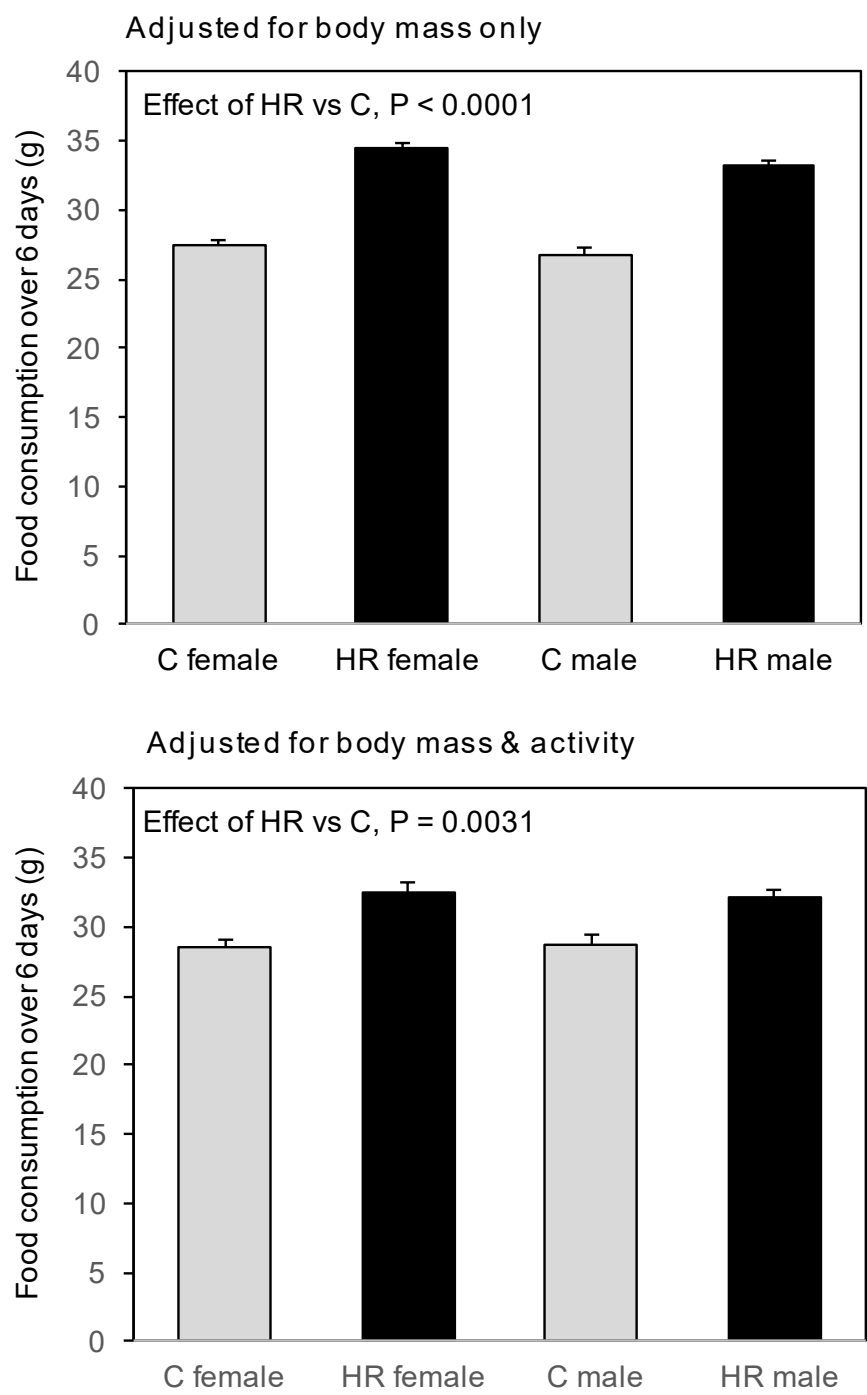


Fig. 2. Food consumption over 6 days of wheel access, adjusted for body mass and activity metrics. HR mice ate more food than C mice, even accounting for their increased activity levels. Activity metrics used as covariates were duration and intensity of wheel running and home-cage activity. Values are least-squares means + 1 standard error from analyses of covariance in SAS Procedure Mixed. Corresponding *P* values are in Table 1. Analyses included covariates of age and age-squared. Each point represents ~80 mice.

3.3. Activity levels

HR mice ran for significantly more distance (revolutions per day) than C mice ($p < 0.0001$) on days 5+6 of wheel access by running more minutes per day ($p = 0.0480$) at higher mean ($p < 0.0001$) and maximum speeds ($p < 0.0001$, Table 1 and Fig. 3). Females ran more than males ($p = 0.0061$) by running more minutes per day ($p = 0.0005$) but not at significantly higher speeds compared with males (Table 1 and Fig. 3).

Total home-cage activity during days 5+6 of wheel access was not different among groups (Table 1 and Fig. 4). Interestingly, females were active more minutes per day ($p = 0.0013$) but at lower intensities ($p = 0.0321$) compared with males (Table 1 and Fig. 4).

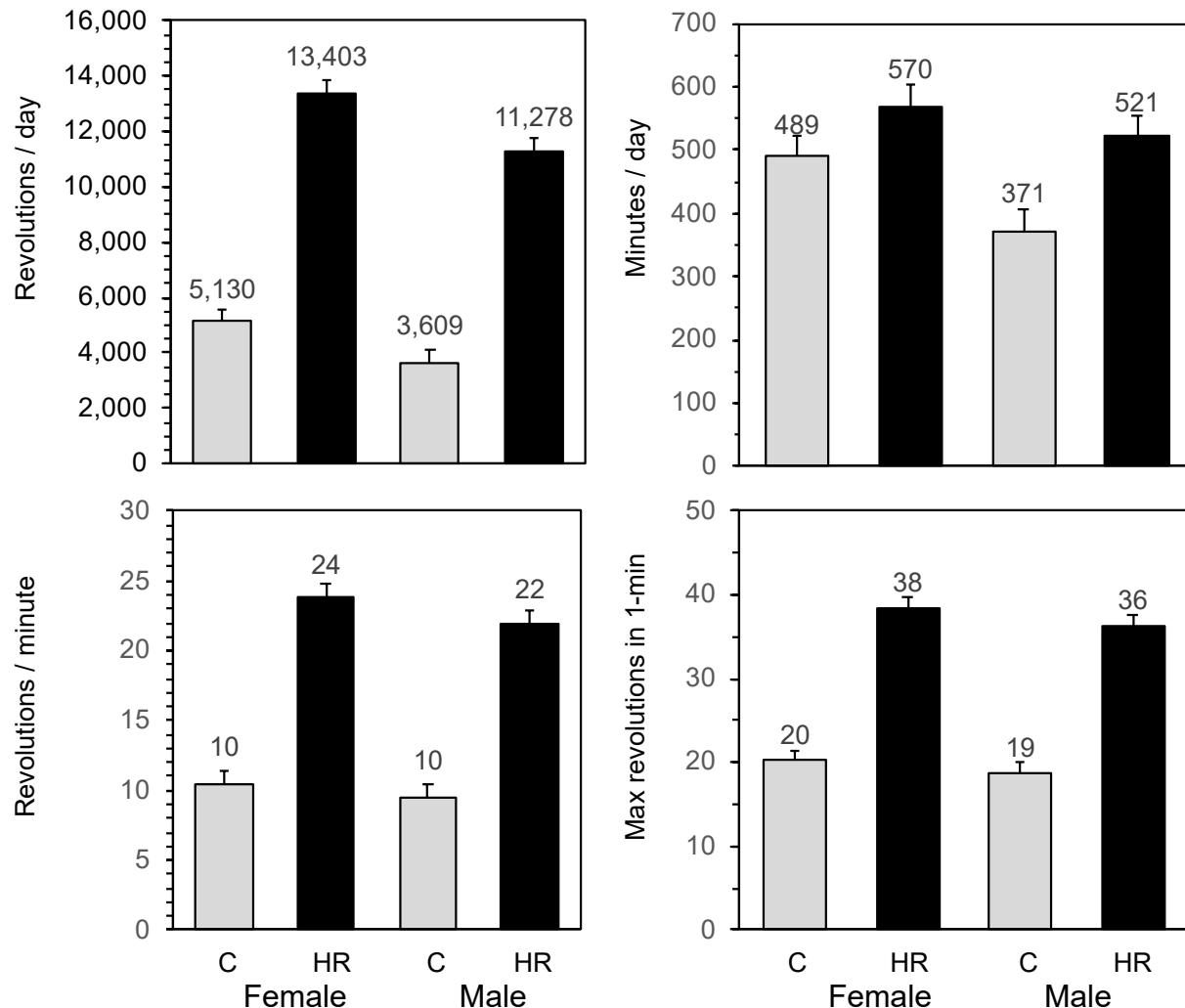


Fig. 3. Wheel running and component traits on days 5+6 of a 6-day wheel test. HR mice ran 2.6-3.1 times more than C by running for more minutes per day and at higher speeds. Females ran more than males by running for more minutes per day. Values are least-squares means + 1 standard error from analyses of covariance in SAS Procedure Mixed. Corresponding P values are in Table 1. Analyses included covariates of age and wheel freeness. Each point represents ~80 mice.

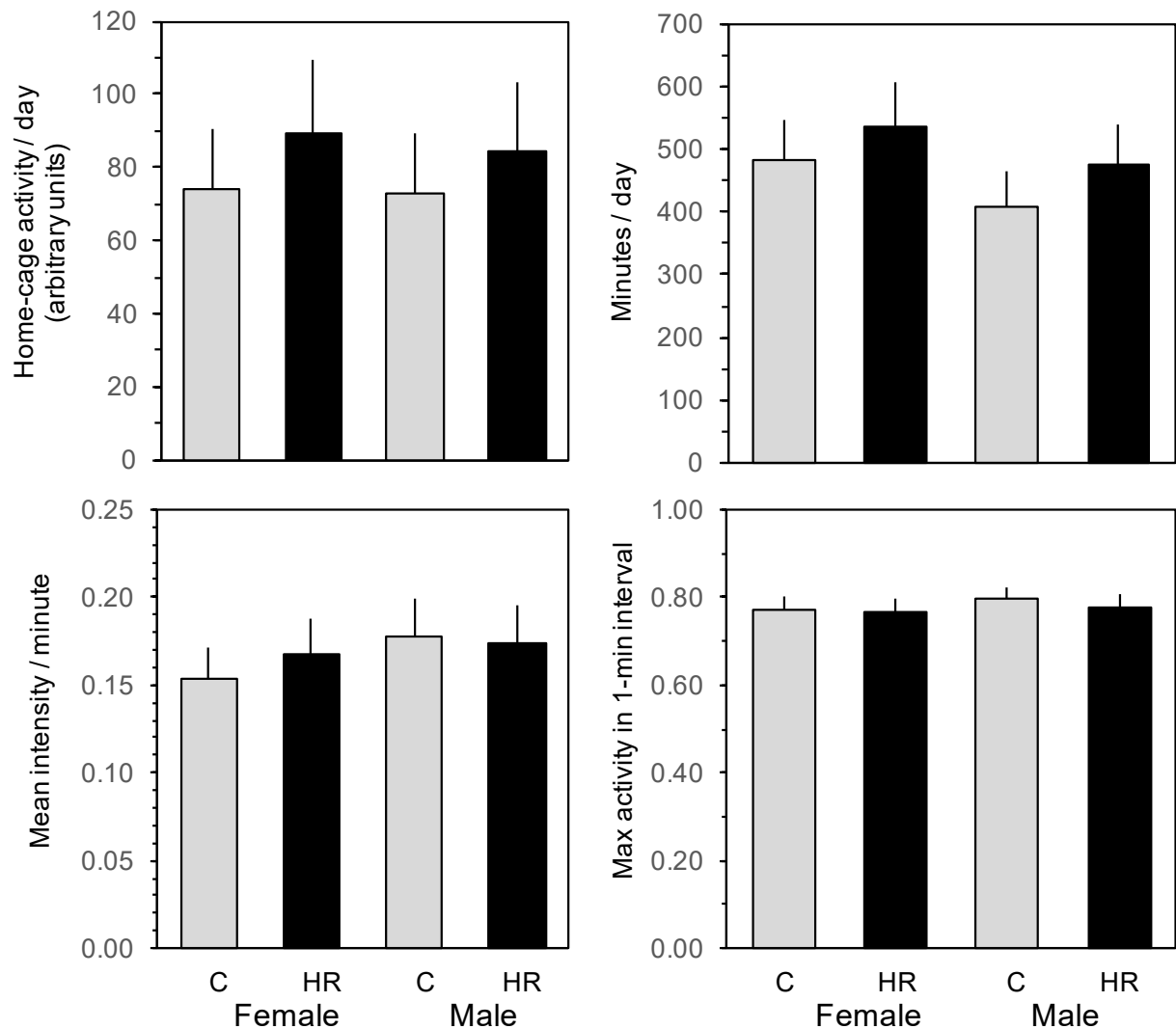


Fig. 4. Home-cage activity and component traits on days 5+6 of a 6-day wheel test. HR were not more active in their home-cages than C. Males had lower duration but higher intensity of home-cage activity compared to females. Total activity, duration, and intensity were log₁₀-transformed and maximum activity was square-rooted before analyses of covariance in SAS Procedure Mixed. The values presented here were back-transformed. Error bars represent the back-transformed upper 95% confidence interval calculated from the mean and standard error of transformed values. Corresponding *P* values are in Table 1. Analyses included covariates of age and sensor sensitivity. Each point represents ~80 mice.

3.4. Structural equation models

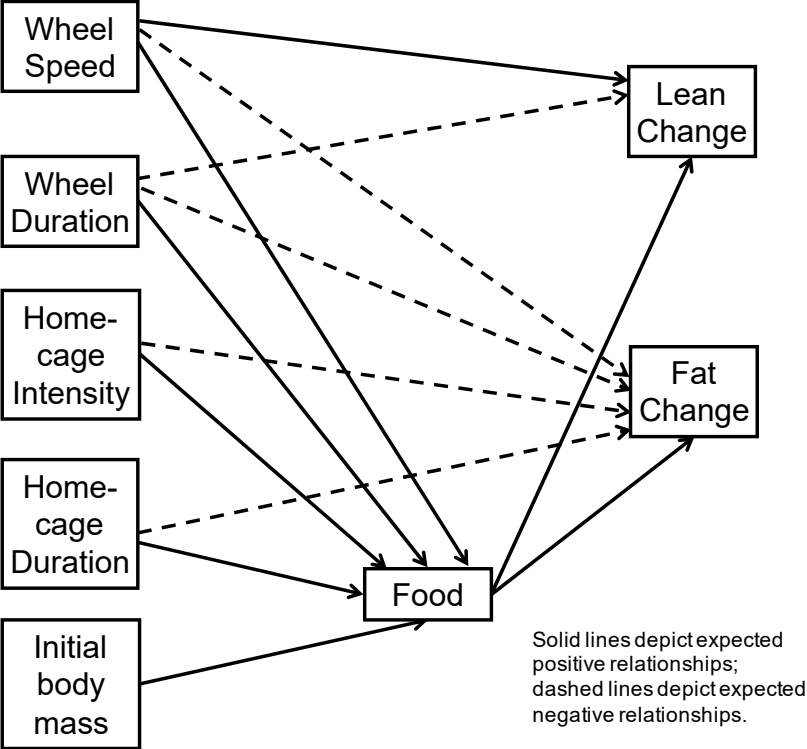
Acute changes in physical activity can lead to energy imbalances that affect body composition, even with *ad libitum* food availability. We used structural equation models to determine the relative importance of different types of activity, initial body mass, and food consumption on lean and fat mass change. The model was analyzed separately for each sex-by-linetype group (i.e., C females, C males, HR females, and HR males) in order to detect differences in phenotypic architecture.

For all 4 groups, the intensity and duration of home-cage activity were positively related and food consumption decreased amount of fat lost over 6 days of wheel access (Fig. 5B, note that all groups lost fat mass, so a positive relationship indicates reduced fat loss). The only other paths shared by all groups were non-significant effects (e.g., wheel speed did not predict change in lean mass in any group, Fig. 5B). As expected, some paths were linetype-specific (e.g., in C but not HR lines, wheel-running speed was positively related to wheel-running duration, Fig. 5B) while other paths were sex-specific (e.g., wheel-running speed predicted food consumption in females but not males, Fig. 5B).

Lean change was only affected by wheel-running duration and this effect was only significant in HR females (HR females with higher running duration gained less lean mass, Fig. 5B). On the other hand, change in fat mass was affected by running speed, decreased by running duration, and increased by home-cage duration in males, and decreased by running duration in HR females (note, decreased means more fat lost and increased means less fat lost; Fig. 5B). Interestingly, the effect of running speed on fat change was opposite in sign for C and HR males. That is, C males that ran faster lost more fat, but HR males that ran faster lost less fat (Fig. 5B and Supplemental Figs. S3-S6 for parameter estimates).

Food consumption was significantly increased by initial body mass for all groups except HR females (Fig. 5B; but HR females also had a positive estimate, see Supplemental Figs. S3-S6) and the effect was greater in males than females (higher parameter estimates in males in Supplemental Figs. S3-S6). Food consumption was also increased by wheel speed (females), running duration (C males), and home-cage duration (HR males; Fig. 5B). Intensity of home-cage activity did not affect food consumption in any group (Fig. 5B).

327 **Fig. 5**
328 **A.**



329 **B.**
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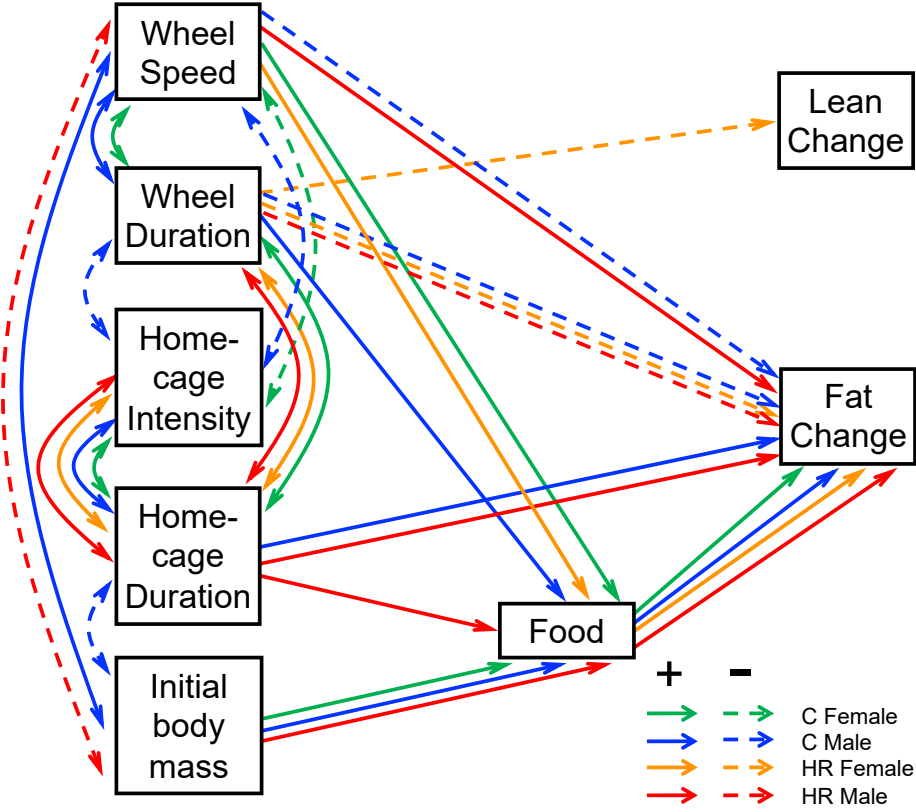


Fig. 5. A: Predicted interrelationships between activity, body mass, food consumption, and body composition (see end of Introduction). Solid lines indicate predictions of positive effects and dashed lines indicate predictions of negative effects. For simplicity, correlations among the components of physical activity are omitted **B:** Structural equation model of activity levels and body mass effects on food consumption and lean and fat mass changes, compiled for 4 groups separated by sex and genetic background. In all groups, food consumption was a significant positive predictor of fat change (within each group, mice that ate more food lost less fat). Overall, path estimates from activity to food consumption were positive. Changes in lean mass were only predicted by amount of exercise in HR females. Changes in fat mass were predicted positively and negatively by activity levels, and 6 of the 7 significant paths were in males. Analyses were run in the structural equation modeling software Onyx. Only significant paths are depicted. Significance was determined by the 95% confidence interval being bound away from zero, which was calculated from the parameter estimates and corresponding standard errors obtained in Onyx. Line color (cyan, blue, pink, and red) represent the four groups by sex and linetype, and style indicates the direction of the effect (solid = positive; dashed = negative). Traits with known or possible differences among replicate lines (activity levels, body mass, food consumption, and mass changes) were centered to have the same mean among the 4 replicate lines within each group. Nuisance variables (age, age², wheel freeness, and sensor sensitivity) were included in the models but are not reported here (see Supplemental Figs. S3-S6). Each group was represented by ~80 mice. The actual parameter estimates for each group can be found in Supplemental Figs. S3-S6.

4. Discussion

Even under *ad libitum* food availability, acute changes in physical activity can lead to energy imbalances that affect body composition. The effect on body composition might also dependent on genetic background and sex, which could have important implications for applying individual medicine in treatments of human obesity and related diseases.

4.1. Among-group differences

Both before and after wheel access, mice from HR lines had lower total body and lean mass compared with C lines (Table 1). These findings are consistent with multiple previous studies on these mice (Swallow et al. 1999, 2001; Copes et al. 2015). Although we did not measure body length in this experiment, previous studies have reported that HR are shorter than C mice (Kelly et al. 2006; Meek et al. 2010; Acosta et al. 2015; Kelly et al. 2017). In addition, HR tend to weigh less than C even when accounting for body length as a covariate (Kelly et al. 2006, 2017), so they are both smaller and skinnier than C mice.

Over 6 days of wheel access, mice of both linetypes and both sexes lost total mass and fat mass, but tended to gain lean mass (Fig. 1). However, mice from HR lines lost significantly less fat than C mice (Table 1, $P = 0.0133$), despite their higher activity in wheels (Fig. 3), suggesting an enhanced ability to conserve fat mass in the face of energetic challenge. At the end of 6 days of wheel access, all groups had approximately 1.7 grams of fat (Fig. 1), or 6% body fat for males and 7.5% body fat for females (Supplemental Fig. S2). This amount of fat potentially represents

a lower limit to healthy adult fat mass in these mice. A previous study also reported fat mass of ~2 grams in C and HR mice after 6 days of wheel access (Hiramatsu et al. 2017).

If a minimum amount of body fat is required to sustain high levels of physical activity over the 6 days of wheel access, then HR mice may be at a limit for activity because of their low body fat. That is, despite compensatory eating, HR mice still lose fat, so they may be unable to increase their activity beyond current levels. This limit in energy balance could be a general explanation for the selection limits experienced in HR lines (Careau et al. 2013).

Food consumption was higher in HR than C mice (with body mass as a covariate; Fig. 2; (also found in Koteja et al. 1999; Swallow et al. 2001)), indicating that HR mice partly compensated for increased energy expenditure by increasing energy intake. Rodent and human studies often report increased food intake to compensate increased voluntary exercise (review in Garland et al. 2011b), although in humans some individuals are “compensators” and others not (see King et al. 2008). The higher food consumption by HR mice was statistically significant even in models that used four separate metrics of physical activity as covariates (Table 1). Thus, in the present study, HR mice ate more than C, even after statistically accounting for their increased physical activity, but they still lost fat. Our finding conflicts with a previous study that found the same four activity metrics could explain the difference in food consumption between HR and C mice (only females tested, Copes et al. 2015; our results did not change when we analyzed the sexes separately [results not shown]). The discrepancy is likely attributable to the fact that the mice they studied were given wheel access for 8 weeks prior to measurements (Copes et al. 2015), which is well after stabilization of wheel running, which occurs after about two weeks in adult mice (Swallow et al. 2001; Acosta et al. 2017).

Several previous studies have reported food consumption of HR and C mice when housed without wheel access, although in most of these the data were analyzed together with mice housed with wheel access, thus making it difficult to see the differences. The overall pattern is that, with body mass as a covariate, HR mice eat somewhat more than C, although the difference is not always statistically significant and the differential may be greater for females than for males.

For example, at generation 10 (Koteja et al. 1999b), adult HR mice of both sexes had higher mass-adjusted food consumption than C lines, but the difference was small and not statistically significant. For adult mice from generation 13, still many generations before selection limits were attained (Careau et al. 2013), Figure 1 in Swallow et al. (2001) shows that HR mice housed with locked wheels (in which HR mice tend to climb more: Koteja et al. 1999a) tended to have greater food consumption than C mice for both sexes, although the differences were only statistically significant at some time points (see also Swallow 1998). Reanalysis of data from Meek et al. (2014) for adult males (last 2 weeks reported) from generation 52 housed without wheels ($N = 50$) indicates that HR mice ate 3.1% more food per day than C mice (linetype $p = 0.4188$, body mass $p < 0.0001$: least-squares means $\pm$ standard errors were 5.01 ± 0.114 g/day for HR mice and 4.86 ± 0.116 for C mice, corresponding to a grand mean body mass of 31.77 g [$SD = 4.241$ g]).

On the other hand, a reanalysis of the food consumption data from Copes et al. (2015) for adult females from generation 57 housed without wheels ($N = 49$) indicates that HR mice ate 13.9% more food per day than C mice (linetype $p = 0.0075$, body mass $p < 0.0001$: least-squares means $\pm$ standard errors were 36.63 ± 0.734 g/6 days for HR mice and 32.16 ± 0.716 for C mice, corresponding to a grand mean body mass of 25.65 g [$SD = 3.529$ g]). Reanalysis of food consumption data from Hiramatsu et al. (2017) for sub-adult males from generation 73, aged 30-

34 days (N = 46) indicates that HR mice ate 1.8% more food per day than C mice (linetype $p = 0.4749$, body mass $p < 0.0001$: least-squares mean $\pm$ standard errors were 3.85 ± 0.069 g/day for HR mice and 3.78 ± 0.086 g/day for C mice, corresponding to a grand mean body mass of 24.64 g [SD = 2.376 g]). For the same mice at age 39-43 days (N = 49), HR mice ate 1.9% more food per day than C mice (linetype $p = 0.3040$, body mass $p < 0.0001$: least-squares mean $\pm$ standard errors were 4.35 ± 0.064 g/day for HR mice and 4.27 ± 0.073 g/day for C mice, corresponding to a grand mean body mass of 24.70 g [SD = 2.326 g]).

It is important to note that neither HR nor C are at a limit with respect to how much food they can consume over these 6 day tests at room temperature. Previously, we found that HR and C mice (of both sexes) can increase food consumption during cold exposure (over 3-6 days) to an average of ~ 10 g per day, which was sufficient to maintain body mass even in ambient temperatures at -15°C (Koteja et al. 2001). In comparison, food consumption during 6 days of wheel access was ~ 4 g in C mice and ~ 6 g in HR mice and all groups lost fat mass (Fig. 5B).

4.2. Structural equation modeling of individual variation within groups

Expected relations among the measured traits are depicted in Figure 5A, and outlined at the end of the Introduction. Only some of these predictions were supported by the data, and we observed a multitude of differences between the sexes and linetypes (Fig. 5B and Supplemental Figs. S3-S6). More specifically, the four sex-by-linetype groups differed in which type of activity (duration or intensity, in wheels or home-cages) significantly predicted food consumption, but overall the path estimates from activity to food consumption were always positive (Fig. 5B), as also reported by Copes et al. (2015). Over the course of six days, we expected that both duration and intensity of physical activity (especially VE) would affect both lean and fat masses; however, changes in lean mass were only predicted by amount of exercise in one group (HR females that exercised for more minutes per day gained less lean mass over 6 days of wheel access).

Although all groups lost body fat across the 6-day period of wheel access (Fig. 1), within each group change in fat mass was positively related to food consumption (Fig. 5B). In other words, individuals that ate relatively more food lost less fat. Perhaps surprisingly, the activity metrics had positive as well as negative effects on fat change, with effect varying among groups (Fig. 5B). Interestingly, six of the seven statistically significant paths from activity metrics to fat change were in males. Future studies will test whether these sex differences might be mediated by differences in circulating hormone concentrations, such as leptin, adiponectin, corticosterone, and the sex steroids.

4.3. Concluding remarks and future directions

Overall, the results of this study suggest that the complex relationships between body size, activity levels, food consumption, and body composition are differentially controlled in the sexes and strongly dependent on genetic background. These potential differences in biological regulation need to be incorporated into studies of the effects of physical activity, especially in human studies where environmental determinants are more commonly assumed (Lightfoot et al. in press). In the HR lines of mice that have been selectively bred for increased exercise, changes to the regulation of energy balance have resulted in a relative conservation of fat mass when faced with acute exercise challenge. However, HR mice still lost fat over 6 days, indicating that the compensation for energy expenditure during high voluntary exercise by increased food consumption (and possibly reduced cage activity: Copes et al. 2015) is incomplete. Furthermore,

the amount of fat that is lost by HR mice may partially explain the selection limit that each of the four replicate lines have reached (Careau et al. 2013).

We chose to do a short-term exposure to voluntary exercise in the present study to mimic procedures used in the selection experiment and hence possibly elucidate the observed selection limits. However, day-to-day increases in wheel running and simultaneous decreases in home-cage activity are still occurring during and after 6 days, with neither measure of activity reaching a plateau until approximately two weeks (Acosta et al. 2017). Thus, an interesting future direction would be to give access to wheels for several weeks and measure changes in body composition as activity levels stabilize. Compensatory behaviors (e.g., increased food consumption, reduced cage activity) may be more or less effective when given longer-term exercise, and may differ between the sexes or linetypes. A related question would be how the starting age of exercise regimes might affect compensatory behaviors and changes in body mass and composition (cf. Jung et al. 2006).

Recent studies have expressed concern that room temperature, being below the lower critical temperature of laboratory house mice, may bias results in some direction (Karp 2012; but see Speakman and Keijer 2012). Some of the heat produced during voluntary physical activity may be used for thermoregulation, thus reducing the net cost of thermoregulation. Interestingly, a previous study on these mouse found that both HR and C mice ran more at 20°C than at 10°C or 30°C (Vaanholt et al. 2006). Therefore, it would certainly be of interest to repeat the present experiments at higher and lower temperatures.

Finally, another approach to the complex interactions of body composition and activity levels would be to study the possible influence of lean or fat mass on activity and food consumption. That is, to explore causality in the opposite direction of structural modeling presented in this study.

As indicated by our results, the phenotypic architecture of obesity-related traits clearly differs between the sexes and in relation to genetic background. Thus, this study lends support for personalized medicine, and points to the need for detailed studies of how different types of prescribed physical activity may or may not be beneficial for regulation of healthy body mass and composition (Drenowatz 2016; Schoeppe et al. 2016; O'Sullivan et al. 2017).

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Supplemental Material

Table S1. Significance levels (P values) from repeated-measures analyses of body mass and composition before and after 6 days of wheel access. Main effects were sex (female or male), linetype (C or HR), wheel access (before vs after access), and all possible interactions of the three main effects. Covariates used were age and age-squared (first standardized and then squared). For lean mass, analyses were run separately by sex and analysis of females did not include age in the model. All statistically significant P values (<0.05) are in bold and signs following the value indicate direction of effect. Sample sizes (N) are approximately doubled because each mouse had two measurements of mass (before and after wheel access).

Trait	N	Sex	C vs HR	After wheels	Age	Age ²	Interactions			
							Sex x C vs HR	Sex x After wheels	C vs HR x After wheels	Sex x C vs HR x After wheels
Total mass	640	< 0.0001 +	0.0567	0.0342 -	< 0.0001 +	0.1955	0.2262	0.8373	0.3261	0.1919
Males	315		0.0397 -	0.1219	< 0.0001 +	0.5600			0.8459	
Females	325		0.1324	0.0515	†	†			0.0587	
Lean mass [†]										
Males	314		0.0468 -	0.0411 +	< 0.0001 +	0.2768			0.0902	
Females	325		0.1371	0.1355	†	†			0.6902	
Fat mass	637	0.7679	0.3510	< 0.0001 -	0.9104	0.8507	0.8649	0.1160	0.0141 -	0.3068
Males	314		0.3697	< 0.0001 -	0.4825	0.1854			0.0043 -	
Females	331		0.3557	0.0013 -	0.6957	0.5284			0.0464 -	

†Unable to estimate due to infinite likelihood. Models with repeated measures for lean mass could not be analyzed for both sexes pooled. Female-specific models for total and lean mass repeated measures could not be analyzed when age or age² was included. Because total mass and lean mass were significantly affected by age, these estimates in the females may be unreliable.

Table S2. Significance levels (P values) from statistical analyses of lean and fat mass percent before and after 6 days of wheel access. Main effects were sex (female or male), linetype (C or HR), and their interactions (“Sex x C vs HR”). Covariates used were age and age-squared (first standardized and then squared). All statistically significant P values (<0.05) are in bold and signs following the value indicate direction of effect. Note, as all mice lost fat, the + sign after the linetype effect (C vs HR) means that HR lines lost less fat mass.

Trait	N	Sex	C vs HR	Sex x C vs HR
Lean mass as percent of body mass				
Before wheel access	333	0.0003 +	0.5488	0.4773
After wheel access	326	<0.0001 +	0.5517	0.4920
Change	320	0.8582	0.0162 -	0.3975
Fat mass as percent of body mass				
Before wheel access	333	0.0041 -	0.2772	0.3745
After wheel access	332	0.0007 -	0.8106	0.4769
Change	322	0.9312	0.0139 +	0.2705

Fig. S1. Scatterplots of total, lean, and fat mass before and after 6 days of wheel access.

Fig. S2. Lean and fat as percent of total body mass, before and after 6 days of wheel exposure. Males had higher lean % and females had higher fat % before and after the 6 days of wheel access. On average, mice gained lean % and lost fat % over 6 days. Values are least-squares means $\pm$ standard errors from analyses of covariance in SAS Procedure Mixed. Corresponding *P* values are in Table 1. Analyses included covariates of age and age-squared. Each point represents ~80 mice. Markers are males = square, females = circle, C = grey and HR = black.

Fig. S3. Structural equation model of activity levels and body mass effects on food consumption and lean and fat mass changes for females from C lines. Analyses were run in the structural equation modeling software Onyx. Thicker lines indicate stronger paths (positive or negative). N ~80 mice.

Fig. S4. Structural equation model of activity levels and body mass effects on food consumption and lean and fat mass changes for females from HR lines. Analyses were run in the structural equation modeling software Onyx. Thicker lines indicate stronger paths (positive or negative). N ~80 mice.

Fig. S5. Structural equation model of activity levels and body mass effects on food consumption and lean and fat mass changes for males from C lines. Analyses were run in the structural equation modeling software Onyx. Thicker lines indicate stronger paths (positive or negative). N ~80 mice.

Fig. S6. Structural equation model of activity levels and body mass effects on food consumption and lean and fat mass changes for males from HR lines. Analyses were run in the structural equation modeling software Onyx. Thicker lines indicate stronger paths (positive or negative). N ~80 mice.

Fig. S1.

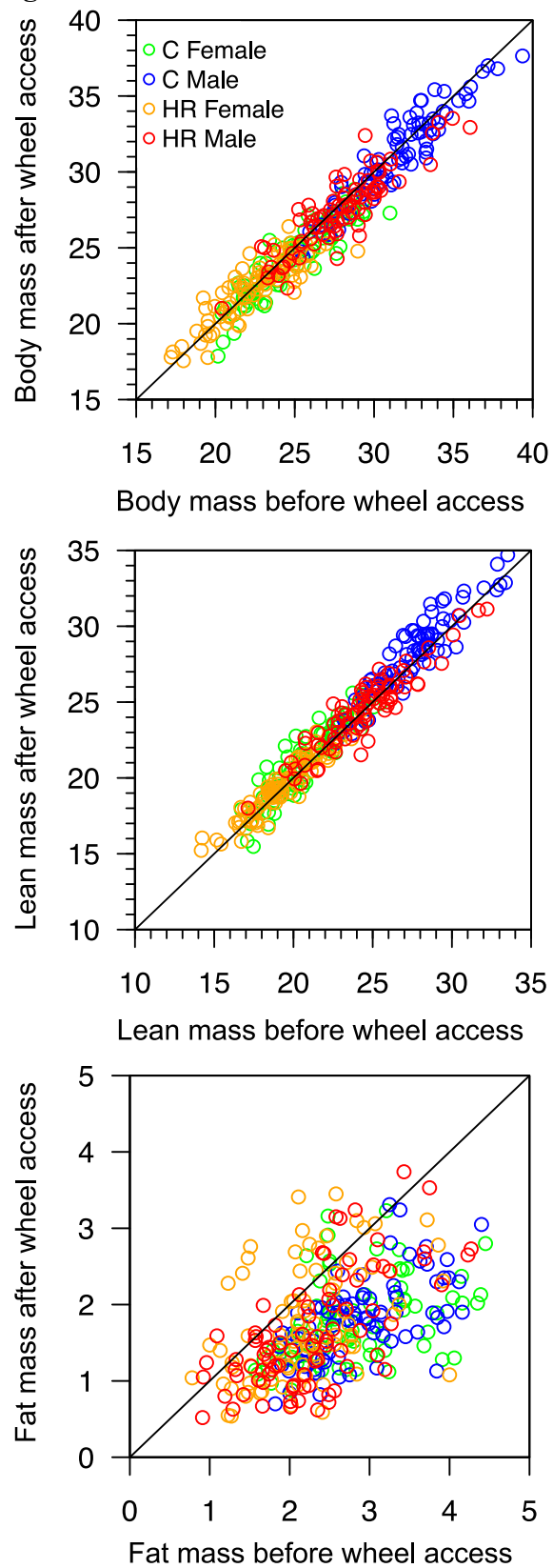


Fig. S2.

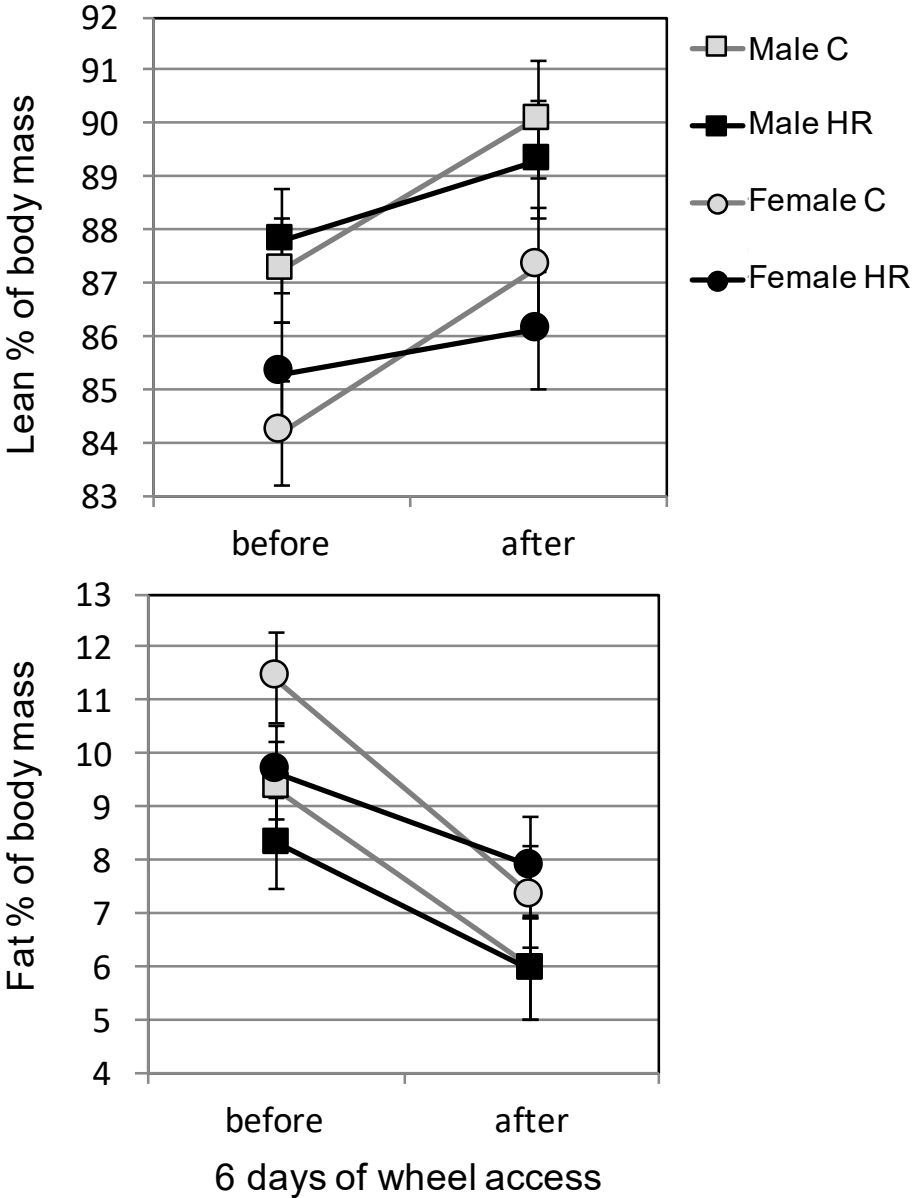
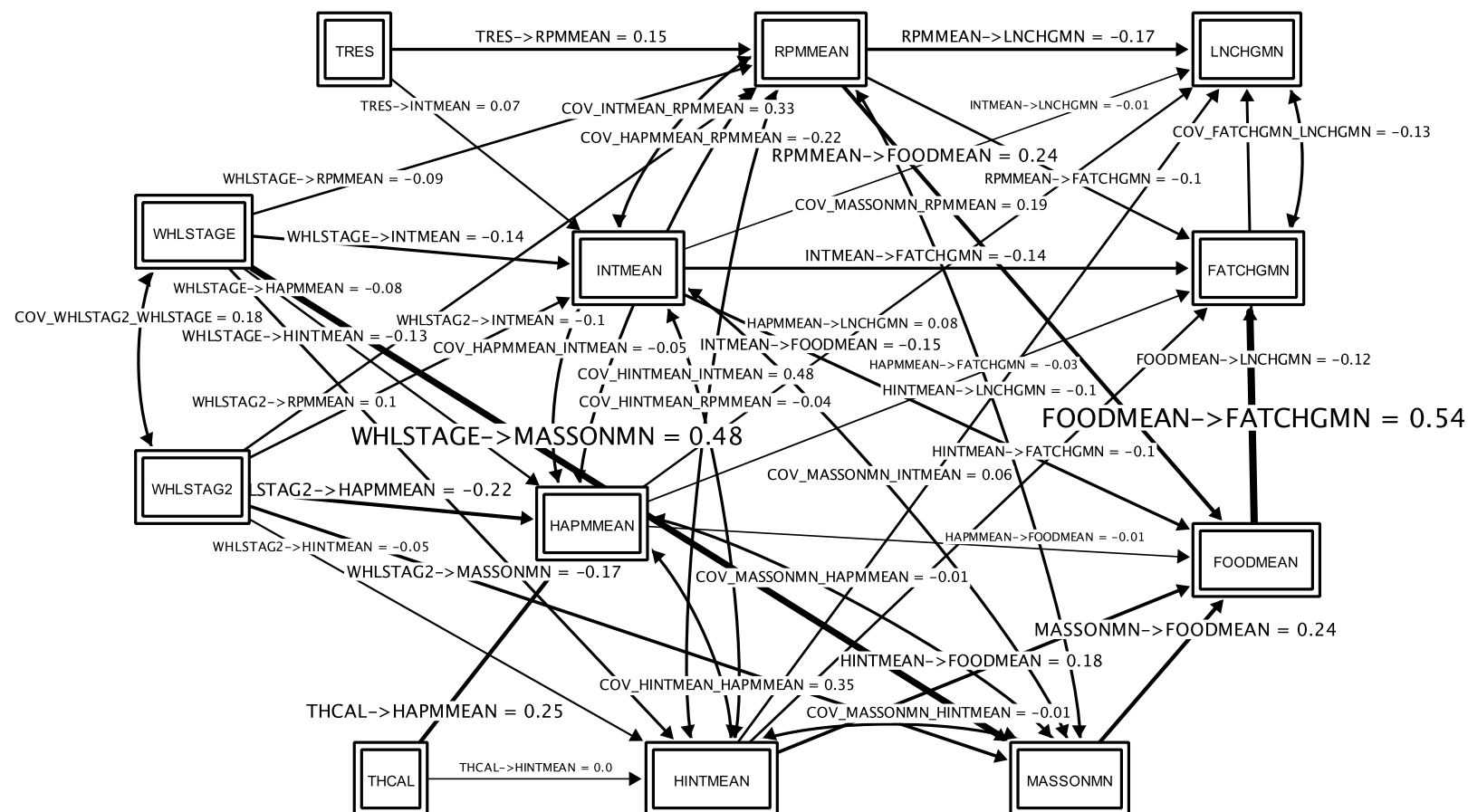


Fig. S3. Structural equation model results from Onyx (standardized partial regression coefficients) – Control females

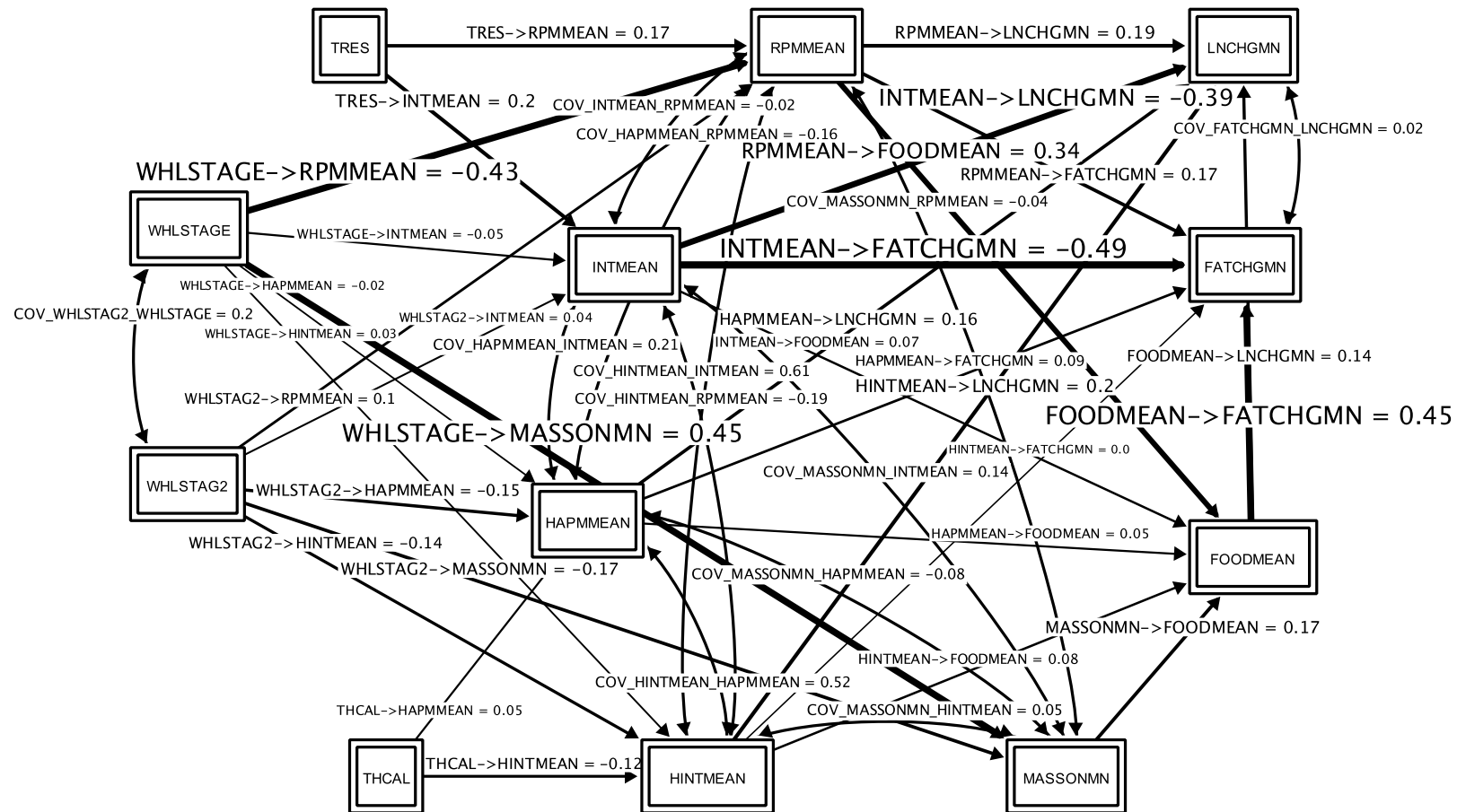


TRES: wheel freeness
WHLSTAGE: age at start of wheel access
WHLSTAG2: standardized and squared age
THCAL: home-cage sensor sensitivity

RPMMEAN: average wheel speed
INTMEAN: wheel duration
HAPMMEAN: average home-cage intensity
HINTMEAN: home-cage duration

LNCHGMN: change in lean mass over 6 days
FATCHGMN: change in fat mass over 6 days
FOODMEAN: food consumed over 6 days
MASSONMN: mass at start of wheel access

Fig. S4. Structural equation model results from Onyx (standardized partial regression coefficients) – High-Runner females

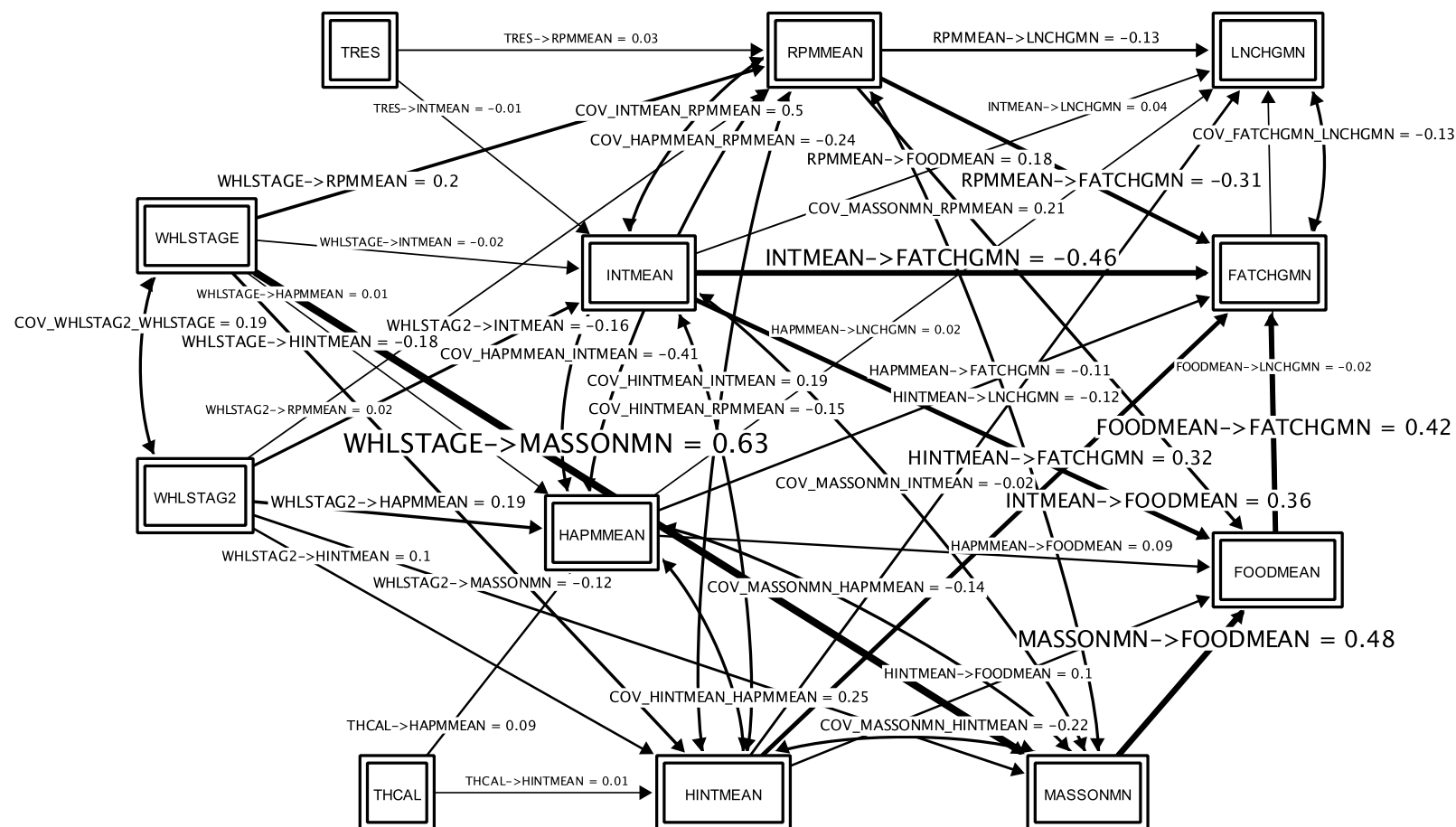


TRES: wheel freeness
WHLSTAGE: age at start of wheel access
WHLSTAG2: standardized and squared age
THCAL: home-cage sensor sensitivity

RPMMEAN: average wheel speed
INTMEAN: wheel duration
HAPMEAN: average home-cage intensity
HINTMEAN: home-cage duration

LNCHGMN: change in lean mass over 6 days
FATCHGMN: change in fat mass over 6 days
FOODMEAN: food consumed over 6 days
MASSONMN: mass at start of wheel access

Fig. S5. Structural equation model results from Onyx (standardized partial regression coefficients) – Control males



TRES: wheel freeness

WHLSTAGE: age at start of wheel access

WHLSTAG2: standardized and squared age

THCAL: home-cage sensor sensitivity

RPMMEAN: average wheel speed

INTMEAN: wheel duration

HAPMEAN: average home-cage intensity

HINTMEAN: home-cage duration

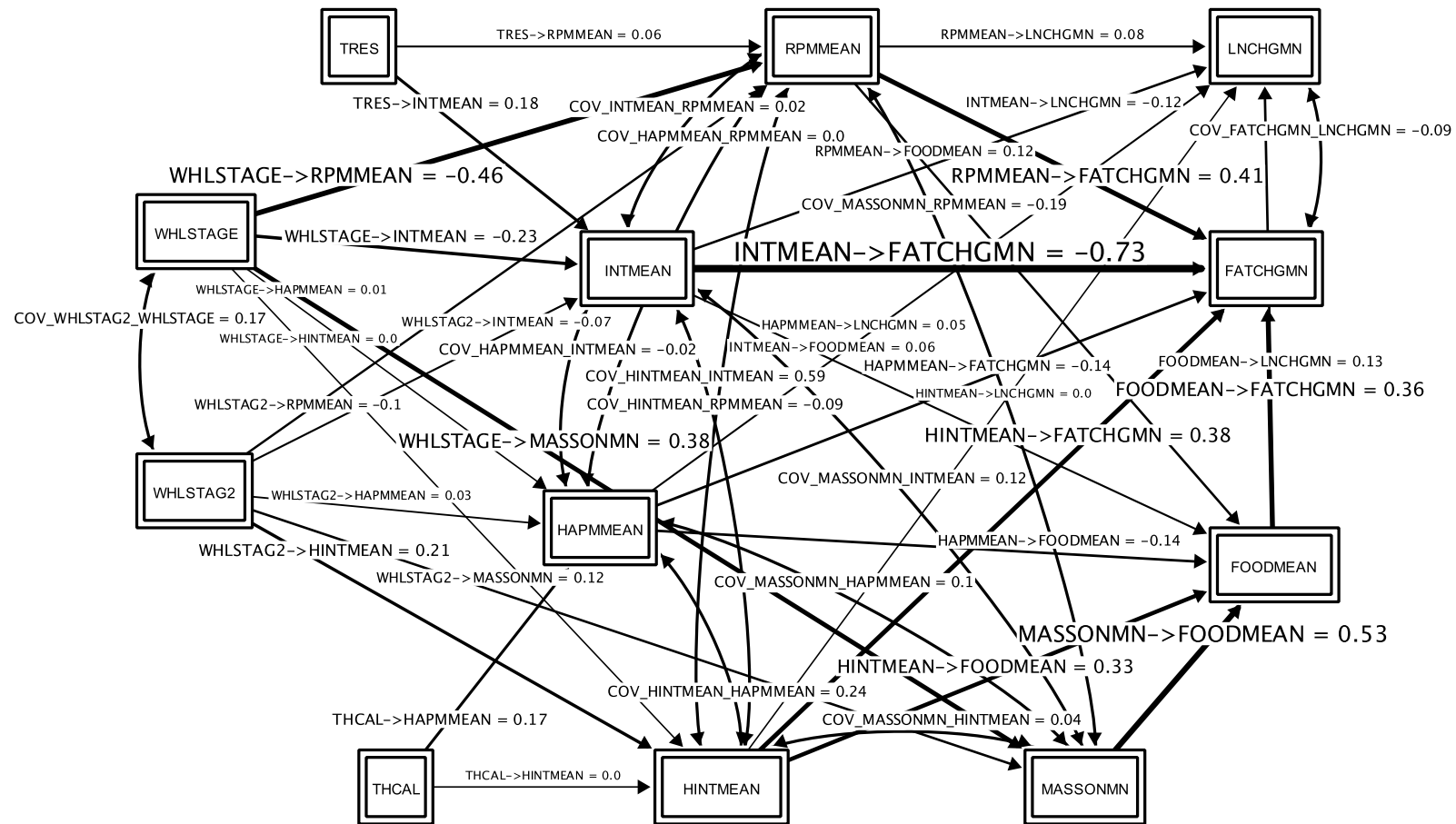
LNCHGMN: change in lean mass over 6 days

FATCHGMN: change in fat mass over 6 days

FOODMEAN: food consumed over 6 days

MASSONMN: mass at wheel access

Fig. S6. Structural equation model results from Onyx (standardized partial regression coefficients) – High-Runner males



TRES: wheel freeness
WHLSTAGE: age at start of wheel access
WHLSTAG2: standardized and squared age
THCAL: home-cage sensor sensitivity

RPMMEAN: average wheel speed
INTMEAN: wheel duration
HAPMMEAN: average home-cage intensity
HINTMEAN: home-cage duration

LNCHGMN: change in lean mass over 6 days
FATCHGMN: change in fat mass over 6 days
FOODMEAN: food consumed over 6 days
MASSONMN: mass at wheel ac

