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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.

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 [18,27,33]. One important cause of energy expenditure is physical activity, the major components of which are voluntary exercise (VE) and spontaneous physical activity (SPA) [10, 41,42], although the definitions of VE and SPA are not always clear (review in [10]). 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 [35] and SPA is recorded by home cage activity [10]. 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., [21]).

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 [19,47,10]. 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 [4,32]). 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 [38]. Mice from HR lines run ~3 times more distance per day than C mice over the 6-day period of wheel access (e.g., [3, 9, 6]) and offer a

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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 [6]. 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 [10,11,46]. For example, HR mice are more active in home-cages when wheels are not provided [7,26], eat more as adults even when housed without wheels [7,40], are smaller in total body mass [21], with the difference more pronounced in males than females [39, 9], have reduced body fat [30,40], reduced circulating leptin levels [12], and increased adiponectin levels [44]. Moreover, the amount of wheel running does not reach a plateau within six days in either HR or C mice (e.g., [1,40]), and neither does the amount of cage activity, a measure of SPA [1], or body mass [5,40]. 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. [19]). Fig. 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 [7] 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 [25]).] 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 [6,38]. 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 [17])). We assigned individuals to wheels balancing by sex and linetype. Wheel running was recorded with an automated counting system in 1-min 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-min interval). Mice were similarly monitored for activity in the home-cage by passive infrared motion-detection sensors [7]. 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-min 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 [38].

We weighed mice and food hoppers (± 0.01 g) before and after wheel access, noting obvious signs of food wasting or shredding [20]. 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 9.4 M4 (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 $\log_{10}$-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 s 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 [45]. 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