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Effects of voluntary exercise on spontaneous physical activity and food consumption in mice: Results from an artificial selection experiment

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HIGHLIGHTS

- Female mice were housed in standard cages or in cages with attached wheels.
- Wheel activity and spontaneous physical activity (SPA) were recorded every min.
- Both duration and intensity of SPA were decreased with wheel access.
- Total activity duration and food consumption were increased with wheel access.
- Both duration and intensity of wheel running and of SPA predicted food consumption.

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ABSTRACT

We evaluated the effect of voluntary exercise on spontaneous physical activity (SPA) and food consumption in mice from 4 replicate lines bred for 57 generations for high voluntary wheel running (HR) and from 4 non-selected control (C) lines. Beginning at ~24 days of age, mice were housed in standard cages or in cages with attached wheels. Wheel activity and SPA were monitored in 1-min intervals. Data from the 8th week of the experiment were analyzed because mice were sexually mature and had plateaued in body mass, weekly wheel running distance, SPA, and food consumption. Body mass, length, and masses of the retroperitoneal fat pad, liver, and heart were recorded after the 13th week. SPA of both HR and C mice decreased with wheel access, due to reductions in both duration and average intensity of SPA. However, total activity duration (SPA + wheel running; min/day) was ~1/3 greater when mice were housed with wheels, and food consumption was significantly increased. Overall, food consumption in both HR and C mice was more strongly affected by wheel running than by SPA. Duration of wheel running had a stronger effect than average speed, but the opposite was true for SPA. With body mass as a covariate, chronic wheel access significantly reduced fat pad mass and increased heart mass in both HR and C mice. Given that both HR and C mice housed with wheels had increased food consumption, the energetic cost of wheel running was not fully compensated by concomitant reductions in SPA. The experiment demonstrates that both duration and intensity of both wheel running and SPA were significant predictors of food consumption. This sort of detailed analysis of the effects of different aspects of physical activity on food consumption has not previously been reported for a non-human animal, and it sets the stage for longitudinal examination of energy balance and its components in rodent models.

1. Introduction

Obesity, and most of its negative health effects, results from complex interactions among physical activity (hereafter, "activity"), diet, environment, sex, genetic predisposition, and socio-cultural factors [1]. Inactivity is thought to be one key factor in the current epidemic of obesity and the metabolic syndrome. The primary components of activity are voluntary exercise (VE) and spontaneous physical activity (SPA). Both VE and SPA can have beneficial effects in humans and animal models (e.g., [2–5]). However, despite decades of study, the relationship

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between activity and energy balance, food consumption, and the regulation of body weight remains controversial [4], in part because humans and other animals may exhibit compensatory behaviors that reduce energy expenditure (or increase energy intake) during other parts of the daily cycle in the face of high levels of activity [6,7]. Whereas many studies emphasize the importance of voluntary exercise per se, others have suggested that SPA (or non-exercise activity thermogenesis, NEAT) can also be crucial for energy expenditure [8,9]. Relatively few studies of human subjects have obtained objective measurements of both types of activity, mainly for practical reasons (e.g., see Colbert and Schoeller [10] and following commentaries). In contrast, rodent models potentially allow relatively easy measurement of both VE via running wheels and SPA in home cages (which may generally equate with NEAT [6,11]). As reviewed elsewhere [6,12], SPA in rodents can be measured using photobeams [13], force plates [14], passive infrared sensors [15], video recording [16] or implanted transmitters [12].

Two specific aspects of VE that remain poorly understood [11] are the main focus of the present study. First, how does VE affect SPA of mice in their home cages? Second, how strongly do VE and SPA affect food consumption? Both are relevant to broader questions concerning motivators of, and constraints on, activity and exercise. For example, a hypothetical constraint based on energy metabolism (e.g., limits to food consumption, processing, or use by active muscles) could cause a negative correlation between VE and SPA, both of which can have substantial energy costs [7,13,17]. A negative correlation would also be expected given temporal constraints (e.g., if time available for activity is limited by requirements for periods of rest or sleep). In addition, if the total amount of activity (or the amount of energy expended in activity) is regulated behaviorally or physiologically, then trade-offs may occur between different types of activity [18]. Conversely, if overall levels of activity are largely governed by motivational factors (e.g., see [19]) then no relationship, or potentially a positive correlation, between VE and SPA might be expected, along with positive correlations between food intake and both VE and SPA. Consequently, the complex dynamics among food consumption, VE, SPA, and adiposity require further investigation, especially given that exercise training can increase both resting energy expenditure and SPA in mice [20].

To examine these issues with the enhanced statistical power that should accompany increased among-group variation in activity, we studied eight lines of mice, four of which have been selectively bred (57 generations) for high levels of voluntary wheel running [21]. Since reaching selection limits around generations 17–25 [22], mice from the four replicate high runner (HR) lines have been running 2.5–3.0 fold more revolutions/day as compared with the four non-selected control (C) lines (e.g. see [17,22]). In addition to this dramatic contrast in wheel running, the HR and C lines have diverged in a range of behavioral, morphological, and physiological traits, a number of which are related to energy expenditure and/or food consumption (e.g. [23,24]; reviews in [19,25]). For example, when housed without access to wheels, HR mice exhibit higher SPA in their normal housing cages [14] and eat more than C mice (adjusting for differences in body mass [26,27]). However, quantitative comparisons of SPA in the presence versus absence of wheels have not been reported (see [28] for an observational study of behavior and activity at generation 10). Moreover, given that SPA is generally reduced in the presence of wheels [11], it is not known if individual variation in SPA contributes to energy requirements (as estimated by food consumption) to a detectable extent in the face of considerable amounts of voluntary exercise. Studies of these relationships in animal models are important because comparable studies of human populations – with equally accurate measures of activity and food consumption – are difficult to implement [4,29]. A particularly striking finding of the selection experiment is the plateau in running in HR lines after generation 16, despite continuing selective breeding [22]. The causal factor(s) for the apparent selection limit are of great interest but remain unclear [17,24,30–32]. No pharmacological manipulation has elicited increased running in HR mice (although a number of agents decreased running behavior (e.g., [19,33]). However, Western diet (high fat, high sugar) causes large increases in wheel running of HR males, with no effect in Control males [29]. Therefore, our second goal was to examine interactions between SPA, wheel running, and food consumption to test whether energetic factors (specifically, expenditure during exercise [as indexed by food consumption]) may prevent further evolution of daily levels of voluntary exercise.

Previous studies indicate that the selection limit in HR mice cannot be explained as a simple constraint on overall energy processing (intake, digestion and absorption, or utilization). Specifically, mice housed in cold environments are able to increase daily energy expenditures substantially above that of HR mice kept at room temperature [34,35(b)], even when room-temperature mice are challenged to work for food by wheel running [35(a)]. However, there could be a limit to the amount of energy useable in skeletal muscles for powering locomotor activity, either VE or SPA. If so, then mice with wheel access would be predicted to show a compensatory reduction in SPA compared to mice without wheel access (i.e., there should be a strong inverse relationship between the amount of VE and SPA, and little or no difference in food consumption in mice housed with versus without wheel access). Given those considerations, we hypothesized that access to wheels would cause an increase in food consumption in both HR and C mice, even though it would also result in decreased SPA in the home cages. In addition, we predicted that the duration and intensity of both wheel running and SPA would be positive predictors of food consumption. Finally, we predicted that the effect of individual variation in wheel running on food consumption would be stronger than the effect of SPA.

2. Materials and methods

Since 1993, four replicate lines of house mice have been bred for high voluntary wheel-running behavior (HR lines), based on the average number of revolutions run on days five and six of a six-day exposure to wheels (1.12 m circumference) attached to standard housing cages [21]. The starting population was 224 individuals from the outbred, genetically variable, Hsd:ICR strain. After two generations of random breeding in our laboratory, mice were randomly divided into eight lines. Four of these were subsequently bred for high running and four were bred without regard to running as Control (C) lines. Each line has been maintained by 10 mating pairs per generation, with occasional exceptions caused by logistical constraints [22]. Sibling-mating is disallowed in all lines.

In generation 57, a total of 100 females from HR and C lines were housed individually beginning at weaning (21 days of age). Apparent food consumption was determined by weekly weighing of food hoppers, with care taken to account for spillage (e.g. see [26,36]). At ~24 days of age, half of the mice were given access to wheels attached to their cages, as in the routine selective-breeding protocol [21]. Each day, we recorded revolutions in each 1-minute interval over a period of 23 h. We then computed the total number of revolutions, the number of 1-minute intervals that had at least one revolution (minutes of wheel activity), and the mean speed of running (revolutions/interval). A measure of wheel freeness was also included as a covariate in analyses of wheel running (e.g. [31,37]). This was measured by accelerating each wheel to a constant speed and then recording the number of revolutions until it stopped. The procedure was repeated four times and average values were used.

Each of the 100 cages was fitted with a corner-placed, passive infrared sensor (Talon TL-Xpress-A; Crow Electronics, Fort Lee, New Jersey, USA) housed in a wire-mesh protective enclosure. The TL-Xpress has an approximate 90° field of view with a reset time following cessation of motion detection of 1–2 s, and has been used in similar studies [15]. The sensors were connected to a digital I/O board (ICS 2313; ICS Electronics, Pleasanton, California, USA) interfaced to a Macintosh computer with custom software developed by M. A. Chappell. The motion sensor software took readings approximately 3 times/s and recorded sensor status as '1' (movement was detected) or '0' (no movement detected).

A mean value (0–1) was computed for each minute, with zero indicating no activity detected and 1 indicating activity detected during each of the approximately 180 scans per min. Mean values were saved every 10 min. Tests indicated that the sensors would detect even small movements (grooming, etc.) and that noise (i.e. false positives) was very rare. Total home-cage activity (referred to as spontaneous physical activity or SPA) was taken as the sum of all activity over 23 h. We also tallied the number of 1-minute intervals during which any SPA was registered. Finally, we divided total SPA by minutes of activity to estimate mean intensity of SPA, i.e., the average amount of activity per minute when any home-cage activity was occurring. This gives a measure that is analogous to mean wheel speed (see previous paragraph). For analyses of home-cage activity data, a measure of sensor sensitivity was used as a covariate, after first subtracting the mean sensitivity from all values. Sensors were calibrated in a relative way by passing a human hand back and forth inside each cage ten times for an approximate 12–15 s. After completing all 100 cages, the entire sequence was repeated two more times. Values were averaged. Neither wheel freeness nor sensor sensitivity was ever statistically significant (all $P > 0.05$), and so they were not included in the analyses of food consumption.

We present data for wheel running, SPA, body mass, and food consumption during the 8th week of exposure to wheels (or not for the sedentary group). This week was chosen because mice were sexually mature, and at an apparent steady state of wheel running activity and body mass, and no wheel or SPA sensor malfunctions occurred. In addition, eight weeks is a common duration of exercise-training studies of house mice (e.g. [29,38]). We also report body length, body mass, and the masses of the retroperitoneal fat pad, liver, and heart ventricle, determined by dissection at the end of the 13th week.

Following numerous previous studies of these lines of mice, data were analyzed as covariance models in SAS Procedure Mixed, with REML estimation and Type III Tests of Fixed Effects. The primary covariate used was body mass, e.g., for analyses of food consumption and organ masses. Statistical significance was judged at $P \leq 0.05$. Linetype (HR vs. C) and wheel access were fixed effects. Replicate line was a random effect nested within linetype (i.e., individuals from the replicate lines were not pooled within linetypes [C vs. HR] for statistical analyses). Effects of linetype were tested relative to line, with 1 and 6 degrees of freedom, as dictated by the design of the selection experiment. Effects of wheel access and of the linetype-by-wheel access interaction were also tested with 1 and 6 degrees of freedom.

Mini-muscle status (a simple Mendelian genetic trait producing greatly reduced hindlimb muscle mass [24,39–41]) was determined at the end of the study based on a comparison of triceps surae muscle masses in relation to body mass, and was included as an additional factor in all analyses (all mice in HR Line 3 were mini muscle, as were a subset of those in HR Line 6). Previously, mini-muscle individuals were shown to have an elevated cost of transport during voluntary wheel running [40], which could affect their energy (food) requirements. In addition, mini-muscle mice have enlarged internal organs (e.g., [25,31,32]).

Some variables were $\log_{10}$-transformed to improve normality of residuals, the homogeneity of their spread when used as independent variables or linearity of relations with covariates (see Results). For analyses of food consumption, seven alternative statistical models were compared, and the Akaike Information Criterion (AIC) [42] was used to gauge which model best fit the data (smaller AIC values indicate better-fitting models). The AIC is based on information theory and is calculated as -2(ln of the maximum likelihood) + 2(number of parameters). In this formulation, smaller values of the AIC indicate better models. The AIC can be considered analogous to an adjusted coefficient of determination (r^2). Whereas the likelihood or r^2 of a model always improves when additional independent (predictor) variables are added, the AIC or adjusted r^2 is penalized for each additional parameter and so can indicate worse fit when additional independent variables are added.

All procedures were approved by the institutional animal care and use committee (IACUC) at the University of California, Riverside.

3. Results

3.1. Body mass and length

Body mass during week eight of wheel exposure (average of values at start and end of six-day period) was not significantly affected by linetype (2-tailed $P = 0.0667$, with HR tending to be smaller than C), wheel access ($P = 0.3980$) or the mini-muscle phenotype ($P = 0.3841$), and we found no linetype–wheel access interaction ($P = 0.6318$). During week eight, most individuals in all groups gained mass (range = -0.9 to $+3.4$ g; grand mean ± SE = $+0.837 \pm 0.078$). Average mass changes (g) within groups were 0.667 ± 0.108 (Control, No wheels, N = 25), 0.917 ± 0.179 (Control, Wheels, N = 24), 0.763 ± 0.164 (HR, No wheels, N = 24), and 1.00 ± 0.174 (HR, Wheels, N = 25). Body masses at the beginning and end of week eight were highly correlated within each group (r = 0.986, 0.963, 0.950, and 0.911, respectively).

At the end of the 13th week, body mass was not significantly affected by linetype ($P = 0.0939$), wheel access ($P = 0.0923$, with wheel-access mice tending to be smaller), the mini-muscle phenotype ($P = 0.4308$) or the linetype–wheel access interaction ($P = 0.5230$). Results (not shown) were similar when body length was used as a covariate ($P < 0.0001$). Body length itself (log-transformed) was not significantly affected by any factor, although HR mice tended to be shorter than C mice ($P = 0.1011$).

3.2. Organ masses

As shown in Fig. 1 (top panel), fat pad mass (log transformed and with log body mass as a covariate, $P = 0.001$) was reduced by chronic wheel access ($P = 0.0370$), but was not significantly affected by linetype ($P = 0.3400$), the mini-muscle phenotype ($P = 0.1691$) or the linetype–wheel access interaction ($P = 0.4423$). Results (not shown) were similar when body length was used as a covariate instead of body mass.

Liver mass (log transformed and with log body mass as a covariate, $P < 0.0001$) was unaffected by wheel access ($P = 0.2791$) or linetype ($P = 0.1113$), but was larger in mini-muscle individuals ($P = 0.0044$), with no linetype–wheel access interaction ($P = 0.2177$). Results were similar when body length was used as a covariate instead of body mass, except that the effect of mini-muscle was not significant ($P = 0.3744$).

As shown in Fig. 1 (bottom panel), heart ventricle mass (log transformed and with log body mass as a covariate, $P < 0.0001$) was increased by chronic wheel access ($P = 0.0483$), larger in HR than C mice ($P = 0.0028$), somewhat increased by the mini-muscle phenotype ($P = 0.0993$), and with no linetype–wheel access interaction ($P = 0.4808$). Results were similar when body length was used as a covariate instead of body mass, except that the effect of wheel access became statistically non-significant ($P = 0.0884$).

3.3. Wheel running and spontaneous physical activity

Individual variation in wheel running (revolutions/day) was quite consistent across days, with the day-to-day correlation ranging from 0.774 to 0.936 (mean = 0.882, N = 49, all $P \ll 0.0001$). Individual variation in total home-cage activity was slightly more consistent across days, with the day-to-day correlation ranging from 0.877 to 0.958 (mean = 0.932, N = 98).

Fig. 2 presents wheel-running activity for mice during week eight of the experiment. Consistent with results from previous generations of

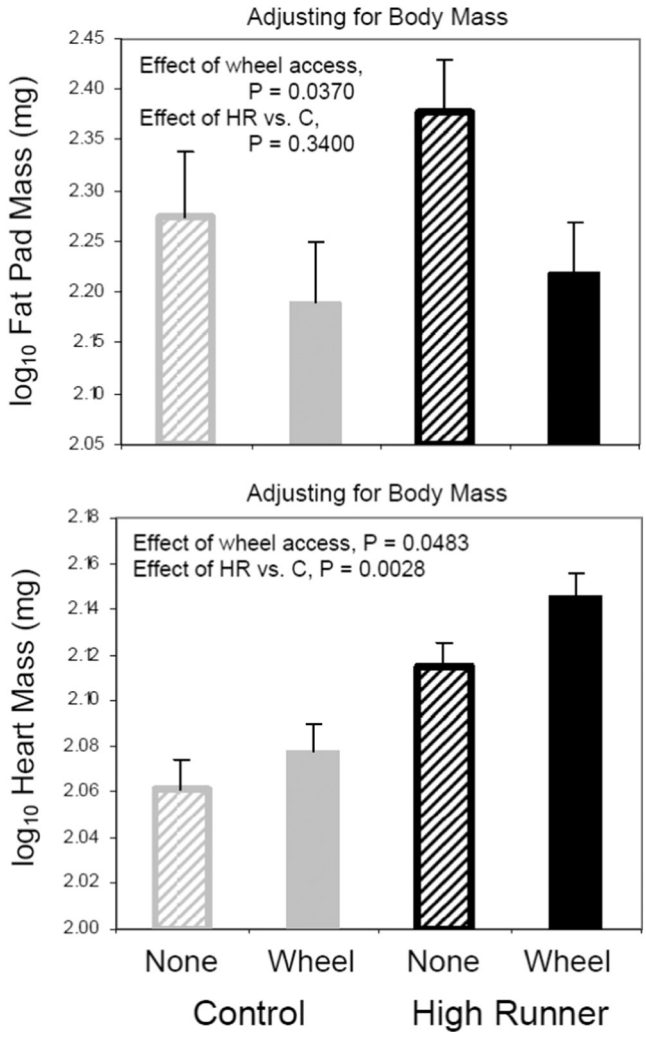

Fig. 1. Some organ masses were affected by long-term wheel access and/or differed between selectively bred High runner and Control lines of mice, after adjusting for variation in log body mass (N = 97). Values are least squares means plus one standard error from nested analysis of covariance models in SAS Procedure Mixed (see text for details). Fat pad mass (top panel) was reduced by chronic wheel access ($P = 0.0370$) in both High runner and Control lines of mice. Heart ventricle mass (bottom panel) was increased by wheel access ($P = 0.0483$) and was larger in HR than C mice ($P = 0.0028$). See text for further statistical results.

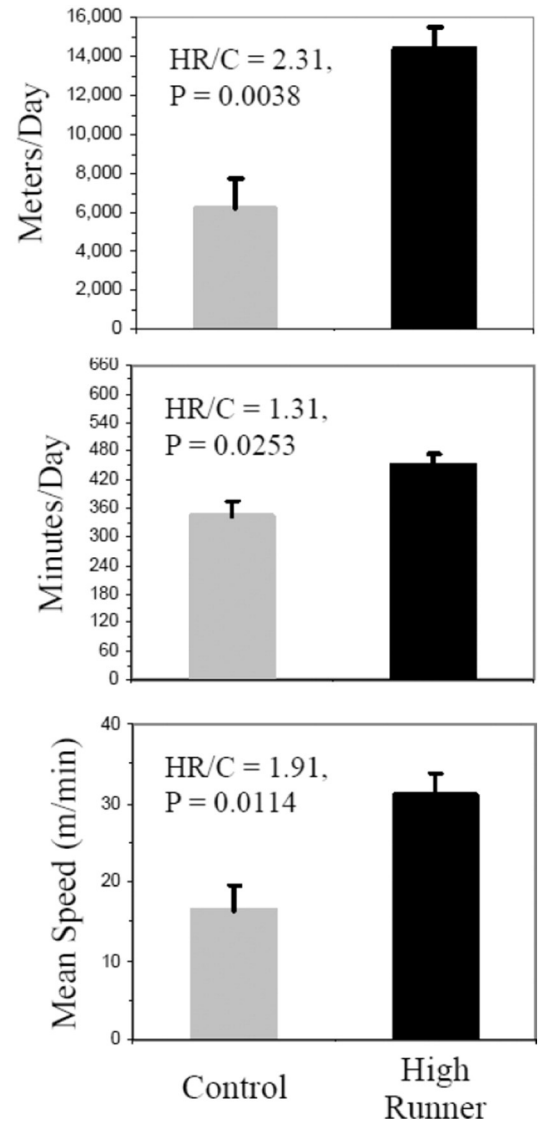

Fig. 2. Mean daily voluntary wheel-running activity of 50 female mice sampled from four replicate High runner lines was greater than for four replicate non-selected Control lines. Values are least squares means plus one standard error from nested analysis of covariance models in SAS Procedure Mixed (see text for details). A measure of wheel freeness for rotation was included as a covariate. Whether or not an individual expressed the mini muscle phenotype was also included as a factor. HR mice ran significantly ($P < 0.05$) more total revolutions, min/day, and at a higher average speed. See text for further statistical results.

the selection experiment (e.g., [14,17,24,31,37]), mice from the HR lines ran more total revolutions than C mice (HR/C = 2.31, $P = 0.0038$), because they ran at higher average speeds (HR/C = 1.91, $P = 0.0114$) and for more minutes per day (HR/C = 1.31, $P = 0.0253$). The measure of wheel freeness was not statistically significant for any aspect of wheel running, nor was the effect of having the mini-muscle phenotype (all $P \geq 0.2$).

As shown in Fig. 3, total cage activity of both HR and C mice was reduced when they had wheel access (log-transformed values: linetype, $P = 0.0276$; wheel access, $P < 0.0001$; interaction, $P = 0.1630$). The reduction was a function of both duration (linetype, $P = 0.1037$; wheel access, $P = 0.0250$; interaction, $P = 0.4931$) and especially average intensity of SPA (for log-transformed values, linetype, $P = 0.0547$; wheel access, $P < 0.0001$; interaction, $P = 0.2528$). The measure of sensor sensitivity was not statistically significant for any aspect of cage activity, nor was the effect of having the mini-muscle phenotype (all $P > 0.18$).

Separate analyses of mice without and with wheels indicated that log-transformed total SPA was significantly higher for HR than C mice when they were housed without wheels ($P = 0.0078$, HR/C = 1.50 for back-transformed least squares means), but not when they had wheel access ($P = 0.2770$, HR/C = 1.13). This differential in total SPA when housed without wheels is somewhat less than the differentials reported for mice measured over only two days using photobeam sensors [43] and over six days using force plate sensors [14]. In terms of SPA duration, HR mice were not significantly more active than C mice either without ($P = 0.1491$, HR/C = 1.16) or with wheels ($P = 0.4117$, HR/C = 1.07). They did have higher log-transformed intensity of SPA when housed without wheels ($P = 0.0258$, HR/C = 1.25 for back-transformed least squares means), although not with wheel access ($P = 0.6116$, HR/C = 1.04).

Total activity during wheel running and in home cages cannot be compared directly because they involve different behaviors, measuring devices, and units of measurement. However, the duration of wheel running and SPA can be compared or summed (e.g. [44]). Wheel access reduced the daily duration of SPA from approximately 8.6 to 7.6 h in C mice, and from 9.9 to 8.2 h in HR mice (Table 1). The corresponding hours of wheel running were 5.7 and 7.5 for C and HR, respectively. Based on previous video analyses, perhaps a third of total wheel

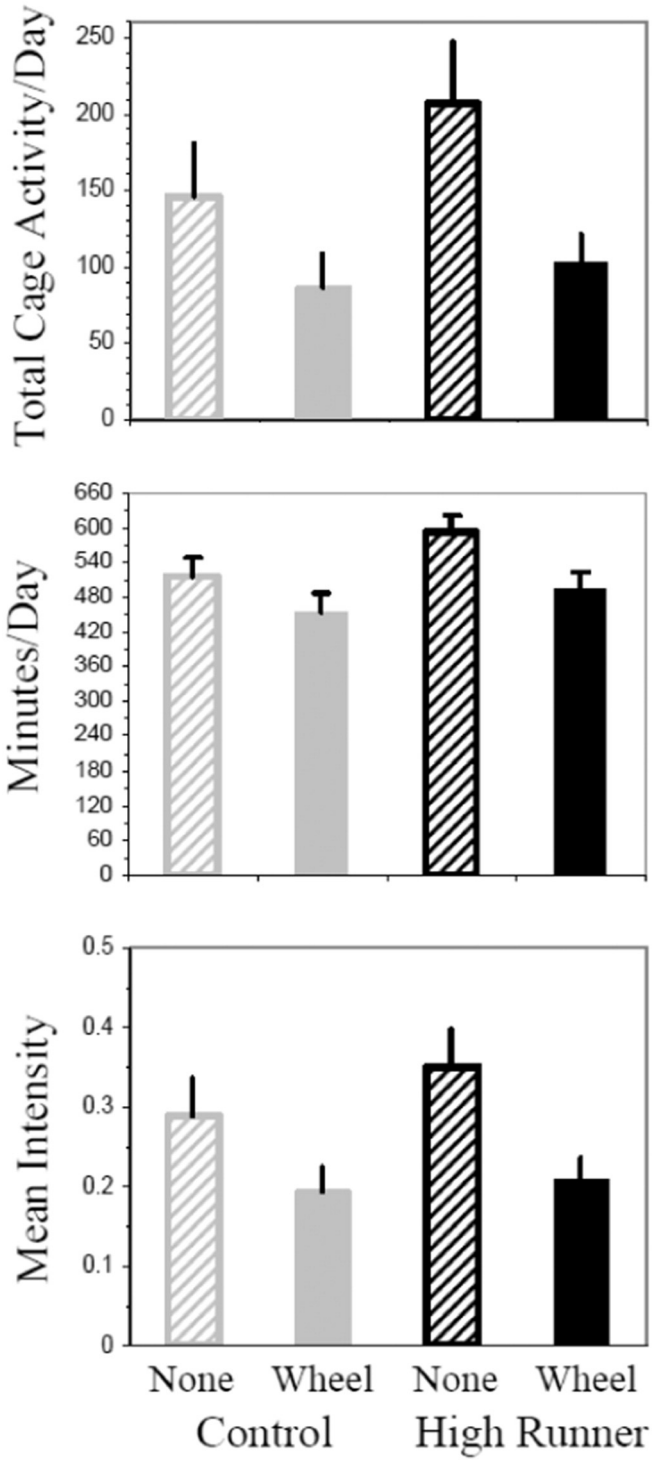

Fig. 3. Mean daily spontaneous physical activity (home-cage activity) of 99 female mice sampled from High runner and Control lines is reduced when they have wheel access. Values are least squares means from nested analysis of covariance models in SAS Procedure Mixed. See text for complete statistical results. Total cage activity (log_{10}-transformed) was significantly higher for HR than C mice when they were housed without wheels ($P = 0.0078$, HR/C = 1.50 for back-transformed least squares means), but not when they had wheel access ($P = 0.2770$, HR/C = 1.13). For middle panel, error bars represent one standard error. For top and bottom panels, values were log_{10}-transformed prior to analyses, and so error bars are upper 95% confidence limits based on back-transformed values.

revolutions are "coasting" (hanging onto a wheel while it rotates) or occur after mice have exited the wheel [28]. Thus, an appropriate conservative estimate decrements those values by 1/3 for comparisons with SPA values, yielding an adjusted duration of wheel activity of 3.8 and 5.0 h, respectively.

Table 1
Time spent in wheel running and in spontaneous physical activity in home cages (h).

Linetype, environment	Control, No wheel	Control, Wheel	High runner, No wheel	High runner, Wheel
Cage activity	8.6	7.6	9.9	8.2
Wheel activity	0	5.7	0	7.5
Total activity	8.6	13.3	9.9	15.7
Adjusted wheel activity	0	3.8	0	5.0
Adjusted total activity	8.6	11.4	9.9	13.2

Values for cage activity and wheel activity are least squares means from nested analysis of covariance models in SAS Procedure Mixed. Total activity was computed as the sum of those values. Adjusted wheel activity represents values reduced by 1/3 to reflect the fact that approximately 1/3 of wheel intervals are "coasting" or occur after mice have exited the wheels, in both Control and High runner mice [28]. Even using adjusted values, total amount of time active is substantially increased when wheels are present.

Combining the duration of home-cage activity with the adjusted duration of wheel running activity, Table 1 shows that the total duration of activity was about 1/3 greater when housed with wheel access for both HR mice (13.2 vs. 9.9 h) and C mice (11.4 vs. 8.6 h). Thus, at least in terms of duration of activity, wheel running (Fig. 1) is partially – but not fully – compensated by a reduction in home-cage activity (Fig. 2).

3.4. Food consumption

Table 2 shows analyses of food consumption using seven alternative models compared using the AIC. For these analyses, mice housed without wheels were assigned values of zero for wheel running and its components. Adjusting only for variation in body mass as a covariate (Fig. 4 top panel), wheel access increased food consumption ($P = 0.0001$) and HR mice ate more than C mice ($P = 0.0072$), with a non-significant interaction ($P = 0.0743$).

When total wheel activity (revolutions/day) and log-transformed total home-cage activity were included as covariates, both were positive predictors of mass-adjusted food consumption ($P < 0.0001$ and $P = 0.0006$, respectively) and the effects of linetype (HR vs. C mice) and wheel access became statistically non-significant (Table 2, Fig. 4 bottom panel). Several other models are presented for completeness, but in the best-fitting model based on AIC, all four measures of activity were positive and statistically significant predictors of food consumption.

For the analysis with components of wheel running and cage activity for only the subset of mice housed with wheel access (Table 3), all four components of activity were positive predictors of mass-adjusted food consumption, but the intensity of home-cage activity was not statistically significant. For mice housed without wheels (Table 4), only the average intensity of home-cage activity was a significant predictor of food consumption.

4. Discussion

Intense voluntary exercise, such as running, typically elicits the highest attainable rates of metabolic power production, and sustained activity can be a substantial fraction of total daily energy expenditure (DEE) in humans and rodents engaging in high levels of voluntary exercise [4,6,26,30,35(a,b)]. Chronic exercise can also lead to various training effects, such as the reductions in fat pad mass and increases in heart mass that we observed here (Fig. 1). These considerations have fueled continued interest in determining (1) how VE affects overall energy budgets, (2) how VE affects other types of physical activity, and (3) the limits to VE. The eight genetically differentiated lines of mice

Table 2
Significance levels from alternative statistical models predicting food consumption (g/six days), with best-fitting model in bold.

	P	*P*	*P*	*P*	*P*	*P*	*P*
Linetype (HR vs. C)	0.0072 [+]	0.0527 [+]	0.0178 [+]	0.2176 [+]	0.0116 [+]	0.0877 [+]	**0.1970 [+]**
Wheel access	0.0001 [+]	0.5668 [+]	0.0002 [+]	0.0950 [+]	0.1926 [−]	0.2533 [+]	**0.0745 [−]**
Linetype × wheel	0.0743	0.2707	0.0349	0.3785	0.7096	0.7487	**0.3357**
Mini-muscle	0.1967 [+]	0.2991 [+]	0.1185 [+]	0.1402 [+]	0.0923 [+]	0.2384 [+]	**0.0402 [+]**
Body mass	<.0001 [+]	<.0001 [+]	<.0001 [+]	<.0001 [+]	<.0001 [+]	<.0001 [+]	**<.0001 [+]**
Wheel revolutions		<.0001 [+]		<.0001 [+]			
Wheel time					<.0001 [+]		**<.0001 [+]**
Wheel mean speed						0.0005 [+]	**0.0045 [+]**
log Home-cage activity			0.0092 [+]	0.0006 [+]			
Home-cage time					<.0001 [+]		**0.0476 [+]**
log Home-cage intensity						0.0800 [+]	**0.0033 [+]**
AIC	536.70	517.05	531.14	505.64	512.34	525.07	**497.44**
Akaike weight	0.000	0.000	0.000	0.016	0.001	0.000	**0.983**

N = 98. Results are from nested analysis of covariance models in SAS Procedure Mixed with categorical factors of linetype (HR vs. C), wheel access, and mini-muscle status; covariates were body mass (mean of values at start and end of 6-day trial) and alternate measures of wheel running and home-cage activity. (For purposes of these analyses, the mice housed without wheels were assigned values of zero for wheel running and its components.) Replicate line was included as a random effect in all analyses (see text for further details). Degrees of freedom for linetype, wheel access, and their interaction were 1 and 6; for mini-muscle status and the continuous-valued covariates, they were 1 and 76 or fewer, depending on the model. [+] indicates direction of effect, including HR > C, wheel access > no wheels, mini-muscle > normal, and positive effect for covariates. AIC is Akaike Information Criterion, with smaller values indicating better-fitting overall model. Akaike weights are relative likelihoods of the models in this set. Least squares means and SEs for the first and last models are shown in Fig. 4.

used in the present study are a particularly advantageous system for investigating these and related questions because the four HR lines have been bred for high VE on wheels and typically run approximately 2.5–3 times as far (revolutions/day) as mice from four non-selected Control lines [e.g., 17,22,29,31,33]. Moreover, when housed without wheels, HR mice have higher SPA [14,28] and eat more than C mice (adjusting for differences in body mass [26,27]). The amount of VE in HR lines has remained stable despite continued selection for tens of generations [17,22,31]. One possible cause of this selection limit is a constraint on total physical activity (wheel running + SPA); if so, then one would predict that SPA should decrease when VE occurs and also that energy intake (i.e., food consumption) should be similar in mice housed with and without wheel access, as has sometimes been reported for standard inbred strains of mice (e.g. [45,46]).

Our results are consistent with the first prediction but not the second. In both HR and C females, SPA was substantially reduced for mice given the opportunity for VE, i.e., access to wheels (Fig. 3). This result is consistent with most but not all (e.g. [45,46]) previous studies of laboratory house mice. Although SPA was reduced in the face of VE, food consumption was significantly elevated in mice with wheel access (Table 2), consistent with previous studies of these mice [26,27]. It is important to note that the present study involves mice that have been acclimated to wheel access for several weeks and have ad lib access to food at all times. Hence, it is not at all like the typical rodent models of "activity anorexia," in which sometimes only a few days of wheel access are provided prior to a food restriction that limits feeding to 1–2 h per day (e.g., [49,50]). In addition, mice in the present study tended to be gaining weight, not losing it [see Results]. Mice in the present study were experiencing acute exercise, which may affect appetite in addition to energy expenditure, but they were in a long-term "maintenance" situation, in which daily exercise is part of the overall, relatively stable, energy balance equation. Thus, in terms of energy requirements, the reduction in SPA that occurs when mice are able to exercise voluntarily (Table 1) clearly does not fully compensate for their increased VE-related exercise expenditure. Moreover, both VE and SPA were significant positive predictors of food consumption at the level of individual variation, indicating that both are measuring energetically relevant aspects of activity. Therefore, SPA as measured in the present study, qualifies as "physical activity" by the usual definition [4]. However, the effect of VE on food consumption was substantially stronger than the effect of SPA (Table 2). The importance of VE for the energy balance of these mice is further demonstrated by the reduced fat pad mass in all mice that were housed with chronic wheel access, a result consistent with human epidemiological observations and intervention studies, which generally show that exercise training reduces fat mass [4].

Conceivably, a limit to VE could be related to a constraint on the total duration of activity, perhaps because of a need to spend a fixed portion of the circadian cycle in sleeping, resting, eating or other maintenance functions. Consistent with that hypothesis is the observation that increased VE in HR females has been achieved largely by increased running speed, with little change in the duration of VE compared to C mice [17,19,21,24,27,30,31]. In the present study, however, female HR mice did spend more time in VE than C mice (Fig. 2), and other studies have shown that HR males have elevated running duration compared to C mice, as well as higher speeds [17,26,27]. In this scenario, an inverse relationship between VE and SPA is also expected, with little change in total activity duration. High-runner females did exhibit elevated SPA in home cages when deprived of wheels. However, when wheel access was available (Fig. 3), the total amount of time spent in activity (wheels plus cages) was greater (by about 1/3) for both HR and C lines compared to mice housed without wheels (Table 1), a finding that argues against a simple time constraint on duration of total activity. Similarly, in young adult humans the amount of non-training physical activity is generally not affected by training activity (and, moreover, total daily energy expenditure is increased by training activity, as with both HR and C mice; e.g. see [6]).

Our results are consistent with previous reports for the HR and C lines, in which wheel access causes increased food consumption for both linetypes (e.g. [48]) and with numerous studies showing elevated caloric intake in human athletes. However, some inbred strains of mice have been reported *not* to show a significant increase in food consumption with (prolonged) wheel access, perhaps because they do not run much [45,46] or possibly because SPA is particularly intense or prolonged in the absence of wheel access, although that seems unlikely. Hence, studies of human energy balance should be cognizant of the extent to which mouse strains and experimental protocols effectively model relationships typically observed in humans. Our monitoring of fat pad and heart mass shows that for our strains of mice, the amount of wheel running was sufficient to elicit classic "training responses" related to exercise physiology (Fig. 1). We encourage future studies to monitor these organs as simple indicators of whether experimental animals exercised enough to be able to detect effects on food consumption and other energy-related traits. Our finding of larger hearts in female HR mice on a body-mass adjusted basis confirms a previous report and is consistent with their elevated maximal aerobic capacity during maximal forced treadmill exercise (VO_2max: [31]).

Previously, we have shown that the time spent running on wheels has a stronger effect on energy costs, and hence food consumption, than the average running speed [27], presumably because the fixed 'postural' cost of running (the elevation above resting metabolism of

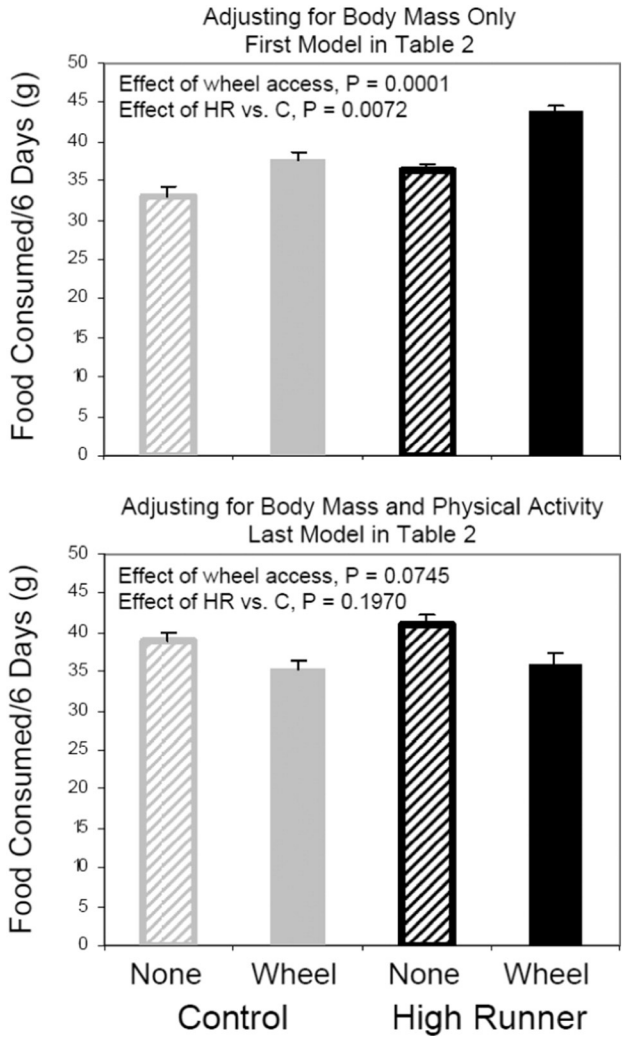

Fig. 4. Food consumption of mice adjusted for variation in body mass is increased by wheel access, and High runner mice eat more than Controls (top panel). Adjusting also for the four quantitative measures of wheel running and home-cage activity (both duration and intensity) eliminates the statistical effects of wheel access and linetype (bottom panel). Values are least squares means and standard errors from nested analysis of covariance models in SAS Procedure Mixed, as indicated in the first and last columns of Table 2, respectively. In the former model, wheel access increases food consumption ($P = 0.0001$) and HR mice eat more than C mice ($P = 0.0072$). In the latter model, the difference between HR and C mice is no longer statistically significant ($P = 0.1970$), indicating that the higher consumption by HR mice can be explained quantitatively by their higher activity levels. The first model (top panel) used to compute group means was (±standard errors): Food consumption (g/6 days) = 20.8273 (±3.5951) + 0.9388 (±0.1449) * body mass − 6.2564 (±1.3487) if C mouse, −7.5065 (±0.9879) if no wheel access, +3.0213 (±1.4004) if C mouse without wheel access, and +1.6518 (±1.2688) if mini-muscle individual. The second model (bottom panel) used to compute group means was (±standard errors): Food consumption (g/6 days) = 7.8796 (±4.5737) + 1.0000 (±0.1084) * body mass − 0.5770 (±1.3335) if C mouse, +5.1915 (±2.5062) if no wheel access, −1.5783 (±1.5085) if C mouse without wheel access, and +1.7997 (±0.8624) if mini-muscle individual + 0.02321 (±0.00488) * wheel time + 0.19060 (±0.0652) * wheel speed + 0.005656 (±0.002809) * home-cage time + 10.1518 (±3.3499) * $\log_{10}$ home-cage intensity.

the zero-speed intercept of the speed versus energy cost regression) is large relative to the speed-dependent incremental cost of running [30,40,52,53]. Therefore, we repeated the statistical analysis with both VE and SPA broken into their components (duration and intensity). As expected, the duration of wheel running has a stronger effect than the average running speed (Table 2). In contrast, for SPA, the intensity of activity had a stronger effect than duration (Table 2). To our knowledge, this sort of detailed analysis of the effects of different aspects of activity on food consumption has not previously been reported for a small

Table 3
Significance levels from statistical analysis of food consumption (g/six days) in relation to physical activity for mice housed with wheel access only.

	P
HR vs. C	0.7138 [+]
Mini-muscle	0.2612 [+]
Body mass	<.0001 [+]
Wheel time	0.0009 [+]
Wheel mean speed	0.0032 [+]
Home-cage time	0.0459 [+]
log Home-cage intensity	0.4422 [+]

N = 49. Results are from nested analysis of covariance models in SAS Procedure Mixed with categorical factors of linetype (HR vs. C) and mini-muscle status; covariates were body mass (mean of values at start and end of 6-day trial) and measures of wheel running and home-cage duration and intensity of activity. Replicate line was included as a random effect in all analyses (see text for further details). [+] indicates direction of effect, including HR > C, wheel access > no wheels, mini-muscle > normal, and positive effect for covariates.

mammal. Extrapolating to human populations in which individuals exhibit a broad range of voluntary exercise (i.e., not just sedentary or clinically confined populations), our results may suggest that exercise levels will be a stronger predictor of energy requirements (food intake) than will levels of non-exercise activity thermogenesis. As noted in the Introduction, few studies of humans have obtained objective measurements of both types of activity, which enhances the potential utility of results from rodent models.

An important new finding of our study is that female HR mice were no more active than C mice in home cages when they had access to wheels (Fig. 3). This demonstrates that voluntary exercise and spontaneous physical activity can be decoupled genetically (see also [54] regarding open-field activity in these lines of mice, and [11] and [55] for general reviews of the issue). Similarly, a line of mice bred for 10 generations for high home-cage activity on days 5 & 6 of a 6-day test (the same as in our selection experiment) ran on wheels no more than might be expected from random genetic drift in comparison with a non-selected control line [56]. In addition, our results indicate that the "hyperactivity" of HR mice (i.e., high relative SPA) can be "treated" by providing the opportunity to exercise. This is consistent with several recent studies showing the efficacy of exercise interventions for treatment of human ADHD (e.g., [57,58]), and also with older rodent studies indicating that wheel running can substitute for other energy-consuming behaviors (e.g., [59]).

A final point of interest is that in our most complete statistical model of food intake (which has a substantially lower AIC than the others), the effect of mini-muscle is positive and significant, even after adjusting for variation in body mass and activity (Table 2). We speculate that the higher food consumption of mini-muscle animals is a function of their

Table 4
Significance levels from statistical analysis of food consumption (g/six days) in relation to physical activity for mice housed without wheel access only.

	P
HR vs. C	0.4953 [+]
Mini-muscle	0.0470 [+]
Body mass	<.0001 [+]
Home-cage time	0.2928 [+]
log Home-cage intensity	0.0004 [+]

N = 49. Results are from nested analysis of covariance models in SAS Procedure Mixed with categorical factors of linetype (HR vs. C) and mini-muscle status; covariates were body mass (mean of values at start and end of 6-day trial) and measures of wheel running and home-cage duration and intensity of activity. Replicate line was included as a random effect in all analyses (see text for further details). [+] indicates direction of effect, including HR > C, wheel access > no wheels, mini-muscle > normal, and positive effect for covariates.

lower efficiency of locomotion on wheels [40]. To our knowledge, this is the first empirical report of an association between locomotor efficiency per se (which is reflected in the efficiency of isolated muscles [60]) and daily energy expenditure in any mammal. In contrast, the higher food consumption of HR mice as compared with C mice is explainable by their greater VE and/or SPA, depending on housing conditions (Table 2).

In closing, we note recently-expressed concerns about the value of rodent wheel running as a model of human voluntary exercise, based on observations that wheel running can induce a variety of behavioral and physiological changes, some acute and others chronic [11]. However, human voluntary exercise also has numerous effects [61,62], and unless and until it is shown that wheel running produces fundamentally different changes than what occur in human voluntary exercise, we will continue to advocate use of running wheels to model voluntary exercise. The HR and non-selected C lines offer unique opportunities for studying the manifold effects of voluntary exercise. When coupled with non-invasive measures of body composition (e.g., [5,63]) measurement systems such as those used in the present study would allow detailed, longitudinal examination of energy balance and its components in rodent models. In such studies, it would be important to use measures of both lean and fat mass as covariates when analyzing food consumption, as these components vary in their maintenance energy requirements and so could affect food consumption. Future studies might also include examination of self-selected exercise intensity [64], sleep (e.g., see [45]), and sedentary behaviors [65].

Competing interest statement

The authors declare no competing financial interests.

Author contributions

L.E.C., H.S., and T.G. designed the experiments. L.E.C., H.S., E.M.D., and W.A. executed the experiments. M.A.C. developed the software for measuring spontaneous physical activity in the home cages. T.G., H.S., and L.E.C. analyzed the data. T.G. wrote the manuscript, with assistance from M.A.C. All authors edited the manuscript.

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