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Food consumption and body composition in mice selected for high wheel-running activity

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Abstract The effects of genetic selection for high wheel running (13th generation) and prolonged access (8 weeks) to running wheels on food consumption and body composition were studied in house mice (*Mus domesticus*). Mice from four replicate lines selected for high wheel-running activity ran over twice as many revolutions per day on activity wheels as did mice from four replicate control lines. At approximately 49 days of age, all mice were placed individually in cages with access to wheels and monitored for 6 days, after which wheels were prevented from rotating for the "sedentary" individuals. During the experiment, five feeding trials were conducted and body mass was measured weekly. After 8 weeks, body composition was measured by hydrogen isotope dilution. Across the five feeding trials, mice in the "active" group (wheels free to rotate) consumed 22.4% more food than mice in the "sedentary" group (wheels locked); mice from the selected lines consumed 8.4% more food than mice from the control lines (average of all trials; body mass-corrected values). In females, but not males, we found a significant interaction between selection and wheel access treatments: within the "active" group the difference in food consumption between selected and control animals was greater than in the "sedentary" group. At the end of the study, mice from the "active" and "sedentary" groups did not differ significantly in body mass; however, mice from the selected lines were approximately 6% smaller in body mass. Estimated lean body mass did not differ significantly either between selected and control lines or between wheel-access groups ($P > 0.3$). Mice from selected lines had lower total body fat compared to mice from control lines ($P = 0.05$; 24.5% reduction; LSMEANS) as did mice from the "active" compared to "sedentary" group ($P = 0.03$; 29.2% reduction; LSMEANS). Under these conditions, a sufficient explanation for the difference in body mass between the selected and control lines was the difference in fat content.

Keywords Body composition · Food consumption · Hyperactivity · *Mus domesticus* · Quantitative genetics

Abbreviation D_2O heavy water

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Introduction

Food consumption (energy intake) and exercise (energy expenditure) are important factors that determine body composition, but genetic background can significantly influence all of these traits and their interactions (Bouchard and Perusse 1994; Bouchard and Shepard 1994). Wheel running, as a model to study effects of spontaneous activity, has been used extensively to study the effects of chronic activity on body composition. Previous research has demonstrated that long-term (greater than 1 month) access to running wheels results in increased food consumption and decreased body fat, with little change in lean body mass in adult mice (Bell and McGill 1991; Bell et al. 1997). A similar pattern has also been

observed in adult rats (e.g., Pitts 1984; Cortright et al. 1997), except that often males will show depressed or no increase in food consumption relative to females (e.g., Tokuyama et al. 1982; Holloszy et al. 1985; Looy and Eikleboom 1989).

The effects of genetic influences on activity, food consumption, and body composition are much less understood and only beginning to be investigated. For example, Lachmansingh and Rollo (1994) and Zachwieja et al. (1997) have looked at the effects of single genes (affecting growth hormone and obesity, respectively) on activity, food consumption, and body composition. We have developed an animal model to study simultaneously the effects of both genetic background and chronic activity on various phenotypic traits (Swallow et al. 1998b). By means of artificial selection for increased number of revolutions run per day in standard activity wheels (Swallow et al. 1998a), we have created lines of mice that after 13 generations of selection run 2.2-fold more revolutions per day compared to controls (mice from non-selected lines: Koteja et al. 1999a).

Artificial selection is increasingly being used as a powerful tool in comparative and evolutionary physiology, in part because selection for a single trait often results in correlated responses in other traits (Garland and Carter 1994; Gibbs 1999). Such correlated evolution is of particular interest because it can reveal underlying shared physiological or biochemical pathways. Significant effects of genetic background have been found in our lines selected for increased wheel running. For example, under various housing conditions and at various ages, mice from the selected lines have been found to exhibit increased $\dot{V}O_{2max}$ (Swallow et al. 1998b), decreased body mass (Swallow et al. 1998b, 1999), decreased retroperitoneal fat pad mass (Dumke et al. 2000), and increased insulin-stimulated glucose uptake in some hindlimb muscles measured in vitro (Dumke et al. 2000). Eight weeks of access to running wheels has resulted in a variety of training responses in both the selected and control lines. These responses include decreased retroperitoneal fat pad mass (Dumke 2000), increased $\dot{V}O_{2max}$, increased respiratory exchange ratio at $\dot{V}O_{2max}$ (Swallow 1998b), and increased skeletal muscle oxidative capacity (Zhan et al. 1999; Houle-Leroy et al. 2000).

Mounting evidence indicated that both wheel access and genetic selection affect body size and aspects of metabolism in house mice. We, therefore simultaneously investigated the effects of artificial selection and wheel access on energy intake and body composition in mice from generation 13 of our ongoing selection experiment (Swallow et al. 1998a; Rhodes et al. 2000). The main question we asked was whether additional exercise (caused by environmental or genetic effects) only affects body mass by decreasing the amount of body fat, or by a combination of changes in lean and fat tissues. The second question we addressed was how environmental and genetic effects influenced energy intake.

Materials and methods

Animal husbandry and breeding design

Outbred, genetically variable Hsd:ICR house mice (Hauschka and Mirand 1973; Carter et al. 1999) (*Mus domesticus* L. Schwartz and Schwartz 1943) were used as the base population for an artificial selection experiment for increased activity. Eight closed populations were established. In four of the lines, mice were selected for high levels of voluntary wheel running; the other four were bred randomly and used as controls. Full details of the selection protocol have been published elsewhere (Swallow 1998; Swallow et al. 1998a, 1998b). Throughout the selection experiment and during the present study, water and food [Harlan Teklad Laboratory Rodent Diet (W) 8604] were available ad libitum, photoperiod was a constant 12:12 h L:D centered at 14:00 hours (Central Standard Time), and room temperature was controlled at approximately 22 °C. Mice were sampled from generation 13, when a more than two-fold difference in activity (average of both sexes) existed between the selected and control lines (see also Swallow et al. 1999; Rhodes et al. 2000). Voluntary wheel running was measured on standard Wahman-type activity wheels, as previously described (Swallow et al. 1998a, 1998b; Koteja et al. 1999a; Rhodes et al. 2000).

Protocol

In the present study, one male and one female were sampled at random from each of the 10 families per line ($n = 160$). After weaning, they were housed in groups of up to four for an average of 28 days (range 20–31 days) in clear polycarbonate cages with wire tops. As part of the routine selection protocol, all 160 mice were placed individually in cages with wheels at 49 days of age (range 41–52 days) and monitored for a 6-day period. At the end of the 6-day period, every other wheel was prevented from rotating by use of a wire tie. Within each family, one individual (either a male or a female) was assigned to a free wheel (“active” group) and the other to a locked wheel (“sedentary” group). Therefore, each of the eight lines was represented by five active males, five active females, five sedentary males, and five sedentary females, with the exception that one sedentary male from a selected line died of unknown causes early in the experiment.

The 159 mice were maintained for the next 7 weeks in the same conditions as described above; as a result, breeding for the selection experiment was also delayed by 7 weeks. Daily wheel running was only recorded over the entire 8 weeks of the study except for days on which animals were manipulated, which occurred 1 day per week (e.g., body mass and food consumption measurements). Body composition was estimated after 8 weeks of the wheel-access treatment via hydrogen isotope dilution (see below).

Food consumption

At the beginning and end of each week, animals were weighed (± 0.1 g). The only exception was during weeks 6–7 (Fig. 1) where the mice were left undisturbed for a period of 13 days and not weighed. Simultaneously, a weighed portion of food [± 0.01 g, Harlan Teklad Rodent Diet (W) 8604] was placed in hoppers. Water was provided ad libitum. Samples of the food were taken to measure dry mass content. Large orts (spilled and leftover food) were collected and dried at 60 °C to a constant mass. Food consumption rate (g/day) was calculated as [(food given×dry mass content) − (dry food uneaten)]/day. Because animals were housed on pine shavings, we were unable to collect the finely crumbled orts. Thus, our food consumption values may be slight overestimates – compare with Koteja et al. (1999b) in which animals were housed on plastic grids and all food and feces were collected and separated manually.

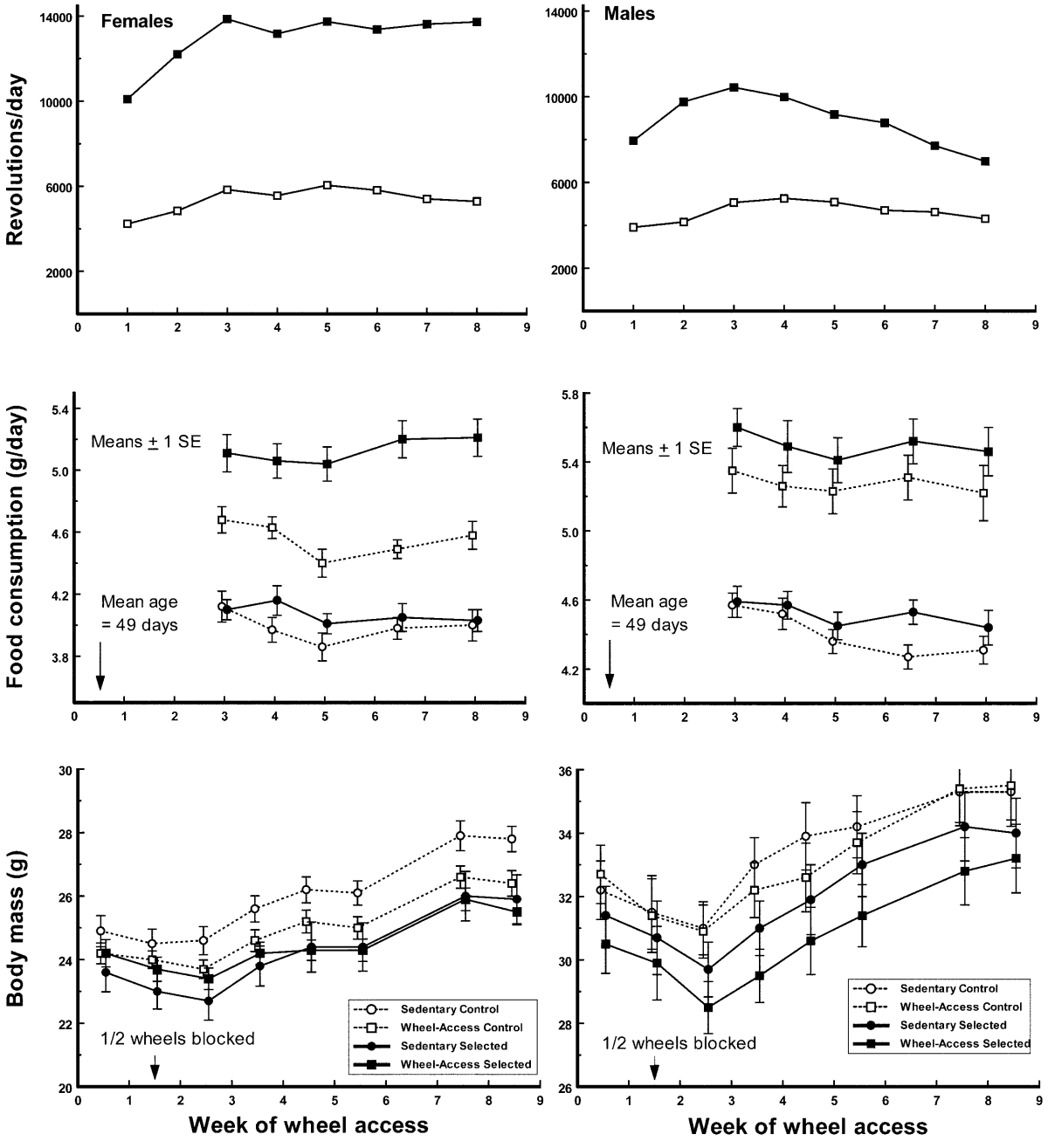

Fig. 1 Rate of wheel activity in revolutions/day (*top*), food consumption (*middle*), and body mass (*bottom*) in house mice with and without access to freely rotating wheels. For half of the animals in the study, wheels were prevented from rotating after 1 week. Food consumption was not measured during the 1st 2 weeks of the experiment. Blood samples used to estimate lean body mass were taken at the end of 8 weeks of wheel running. Values were calculated from the raw data and are presented as weekly means ± 1 SE

Body composition

Because many of the animals in this study were needed to propagate the selected lines (72 of 159 animals used in this study were subsequently used as breeding stock), a nondestructive method of measuring body composition was required. Hydrogen isotope dilution was used to estimate lean body mass. Fat content was then estimated as the difference between body mass (body mass after eight weeks of wheel access) and our measurement of lean body mass [estimated via heavy water (D_2O) dilution].

At 105 days of age (range 97–108 days), after 8 weeks of treatment (wheel-access vs. sedentary housing), animals were injected intraperitoneally with 55.4-µl 99.9% deuteriated water (D_2O; ICN Biomedicals) and then weighed to the nearest 0.1 g. Two blood samples (two microhematocrit tubes, each of ca. 60- to 80-µl volume of blood) were taken from the infraorbital sinus 1 h post-injection. Blood samples were microdistilled, and each sample was analyzed for D_2O enrichment by infrared spectrophotometry following protocols described previously (Karasov et al. 1988; Karasov and Pinshow 1998). Briefly, D_2O enrichment in the distilled samples was measured using a Miran 1FF Fixed Filter Laboratory Analyzer (Foxboro, South Norwalk, CT) with a single wavelength filter factory installed at 4 µm. Approximately 18-µl samples were injected into the top port of the cell (CaF_2 Precision Sealed Cells; Foxboro) with a 10- to 100-µl Eppendorf pipette whose plastic

pipette tip was trimmed by ~3 mm to ensure a good seal with the port. Output from the instrument was interfaced with a personal computer by an analog-to-digital converter and a voltage reading was recorded each second. The output was read for 75 s after sample injection. The inverse of the voltage reading is proportional to water volume. The highest 20 consecutive-second readings (filtered to exclude the highest and lowest second) provided that best predictor of lean body mass. The cell was rinsed three to four times with distilled water and then dried for 40 s with an N_2 jet between samples. Because the instrument was zeroed with distilled water, all measurements of D_2O enrichment reported here are above background.

We used 11 animals (6 males and 5 females) to establish a calibration curve for our indirect measure of body composition. Animals were injected with D_2O and blood samples were taken and analyzed as described above. After the blood sample was taken, the animals were killed by cervical dislocation. Whole animals were freeze-dried for 2 weeks, then ground in a blender with liquid nitrogen. The entire contents were then divided into 2–4 portions of approximately 3–5 g each and placed in extraction thimbles, then dried at 50 °C for 14 h. After weighing, samples were refluxed with petroluem ether for 8 h (Dobush et al. 1985) in a Goldfisch apparatus (Labconco) to measure fat content. For the first batch of six samples, we repeated the extraction procedure for an additional 6 h and found only traces of fat (<0.01 g). Total body fat was calculated as the sum of the extracted fat from all samples for a given animal. Lean body mass was calculated as total body mass (measured after injection of D_2O) minus body fat. Note that lean mass included bone, hair, muscle, viscera, water, and contents of the gastrointestinal tract. For the 11 animals used, body mass ranged from 19.4 g to 36.6 g and total body fat ranged from 0.8 g to 3.7 g.

The calibration curve for predicting lean mass from D_2O enrichment was derived by linear regression. Inverse D_2O enrichment (inverse voltage reading from infrared spectroscopy) was the dependent variable and lean mass was the independent variable, yielding the following equation: $1/\text{IRS reading} = 0.0375 + 0.0460 \times \text{lean mass}$ ($r^2 = 0.9849$). This calibration curve was used to predict lean mass for all animals in our study. Fat mass was then estimated as the difference between body mass and predicted lean mass. Blood samples for a fraction of the individuals injected were lost and omitted from all analyses. In addition, data were omitted for four animals (two males, two females) whose predicted lean mass was greater than their body mass. Thus, the sample for which we have estimates of fat mass was reduced compared to total sample size (see Fig. 5 of Swallow 1998).

Statistical analysis

The General Linear Models (GLM) procedure in SAS was used to estimate three different types of analysis of covariance (ANCOVA) models. First, a cross-nested two-way ANCOVA model was used to test effects of linetype (selected vs. control) and activity group (sedentary vs. wheel-access) on body mass (measured week 3 to end of week 8) and food consumption. Second, a nested one-way ANCOVA was used to test for effects of linetype on body mass at the beginning of the 1st and 2nd week of access to running wheels (i.e., before half of the wheels were prevented from rotating). Similar models were used to test the effect of linetype within a given activity group. For instance, we tested the effect of linetype on food consumption within the wheel-access group. Third, 2-way repeated-measures models were used to test the simultaneous effects of linetype and activity on food consumption across weeks. In the repeated-measures models, between-subject effects (e.g., linetype, activity group) were tested with covariates in the models while within-subject effects (week) were tested with no covariates.

Most of the analyses were performed separately by sex because of the differences in size and activity. However, body composition was analyzed as a combined data set with sex in the model because many previous studies with rats have shown sex differences in body composition and its response to exercise (e.g., Hoyenga and Hoyenga 1982; Pitts 1984).

The two main grouping factors, linetype and activity, were considered fixed effects. Replicate line ($n = 8$ total), nested within linetype, was a random effect. In the foregoing mixed models (i.e., with both random and fixed effects), we tested the effects over appropriate error terms (Sokal and Rohlf 1981; Swallow et al. 1998b).

A variety of covariates were used in the ANCOVA models. Age, time of day, and (z-transformed time of day)^2 were included as covariates in all models of body mass. Time of day is used as a covariate to control for linear changes in mass caused by gastric emptying. Time of day^2 allows for the mass loss to be nonlinear. Z-transformation reduces colinearity with time of day. Age, time of day, (z-transformed time of day)^2, body mass at the beginning of each feeding trial, and body mass change (per day) were included as covariates in all models of food consumption. Body mass change is included in these models to account for energy balance changes across the weeks. In the course of model derivation, plots of raw data and of residuals were inspected. Adjusted means were calculated using the LSMEANS command in the SAS GLM procedure; all covariates in the model, regardless of statistical significance, were used to calculate adjusted means.

A priori, we hypothesized that mice with access to running wheels would consume more food (Koteja et al. 1999b) and have less body fat than sedentary mice (Bell and McGill 1991; Bell et al. 1997). Therefore, we used a 1-tailed test when determining statistical significance of the activity effect (but not of the selection effect) on these two traits. The 1-tailed P values were determined by halving the P values reported by SAS (Sokal and Rohlf 1981). Statistical significance was judged at $P < 0.05$.

Results

Food consumption

Food consumption ranged from 3.04 g/day to 7.00 g/day for individual mice (Fig. 1). In the repeated-measures ANCOVAs for both sexes, food consumption was positively related to body mass prior to the first feeding trial ($P < 0.001$). In the models of food consumption for each individual week, changes in body mass were always significantly positively correlated with food consumption in females ($P < 0.05$), but not in males.

Repeated measures ANCOVA (initial body mass as a covariate) indicated statistically significant effects on food consumption of both activity ($P < 0.001$) and linetype ($P < 0.05$). The activity×linetype interaction was statistically significant for females ($P < 0.01$) but not for males; in females, the difference in food consumption between selected and control mice was greater in the wheel-access group than in the sedentary group. Selected mice with access to functional running wheels had the highest food consumption in all feeding trials; control mice with locked wheels always had the lowest (Fig. 1). Full tables of weekly ANCOVA results are presented in Swallow (1998).

Using the average of the adjusted means from the five feeding trials for activity group (i.e., mass corrected value from LSMEANS for Activity factor), mice with access to freely rotating wheels ate 0.88 g/day (4.87–3.99 g) for females and 1.00 g/day (5.43–4.43 g) for males more than mice with locked wheels. These values represent a 22.1% (females) and 22.6% (males) increase in total food consumption relative to sedentary

individuals. Effects of selection for high activity on food eaten per day were smaller and averaged 0.46 g/day (4.66–4.20 g) for females and 0.35 g/day (5.10–4.75 g) for males. Mice from selected lines consumed 11.0% (females) and 7.4% (males) more food than controls. Unadjusted values for food consumption are shown in Fig. 1. Results for females were unchanged regardless of whether covariates were used. In males, the effect of linetype was no longer statistically significant if body mass was controlled by including it as a variable in the model; the other covariates did not significantly alter the results.

Body mass

At the beginning of wheel access (49 days of age; range 42–52 days), mice from selected lines were, on average, lighter than mice from the non-selected control lines [24.6 g for control vs. 23.8 g for selected (–3.3%) for females; 32.6 g vs. 30.9 g (–5.2%) for males; adjusted means from ANCOVA models split by sex], although the difference was statistically significant only for males. The same pattern was also evident at the end of the experiment [27.2 g for control vs. 25.6 g selected (–5.9%) for females and 35.5 g vs. 33.5 g (–5.6%) for males; adjusted means from ANCOVA models split by sex] at 105 days of age (range 97–108 days). A two-way ANCOVA on final body mass indicated a significant effect of selection only in males ($P < 0.05$) and that wheel access did not cause a significant decrease in body mass for either sex ($P > 0.10$). All mice, both males and females, lost body mass during the 1st several weeks of wheel access (Fig. 1). Because all mice had access to running wheels during the 1st week of this experiment, any effect of wheel access on body mass may have been obscured. Unadjusted values for body mass across all 8 weeks of the experiment are shown in Fig. 1. Results of the analyses are not significantly altered if covariates are removed.

Body composition

The relationship between body mass and predicted lean body mass based on our calibration curve (see Materials and methods) is shown in Fig. 2. Neither linetype nor wheel access significantly influenced lean body mass in either sex ($P > 0.3$). Males, on average, had nearly 0.75 g more fat than females. In females, estimated body fat ranged between 0.5 g and 5.4 g (LSMEANS = 2.75 g). In males, body fat ranged between 1.0 g and 7.0 g (LSMEANS = 3.48 g). Analyses of total fat, either including total body mass as a covariate or expressed as a percent of body mass (Fig. 3), do not show a significant sex effect (Table 1). The ANCOVA including both sex and final body mass shows significant effects of both activity ($P = 0.050$) and linetype ($P = 0.031$); mice with access to functional wheels had reduced body fat compared to mice without (2.65 g vs. 3.51 g), and mice from selected lines had less body fat than did individuals from the non-selected control lines (2.61 g vs. 3.56 g; Table 1).

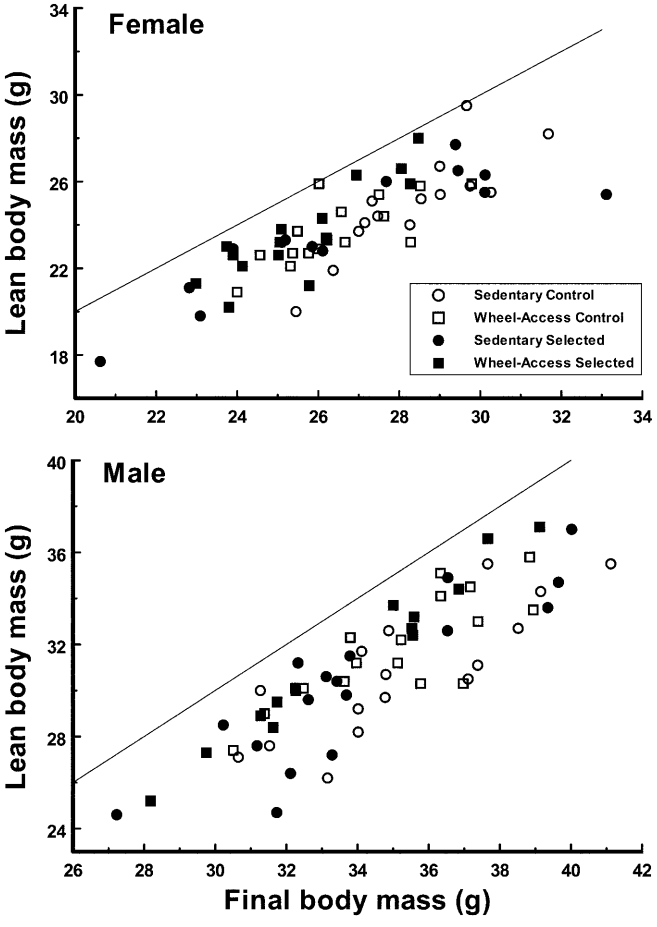

Fig. 2 The relationship between lean body mass (as determined by hydrogen isotope dilution) and body mass for females (*top*) and males (*bottom*). Body mass and lean body mass were measured at 105 days of age, after 8 weeks of wheel-access treatment. *Solid line* represents a 1-to-1 relationship

Discussion

Food consumption

Contrary to models which suggest that the cost of transport should be extremely low in small-bodied mammals living in the wild (Garland 1983), our results demonstrate that the cost of wheel-running activity can represent a substantial portion of the total energy budget of laboratory house mice. Based on our comparisons of mice housed with and without access to freely rotating wheels, wheel running resulted in a 22.4% (LSMEANS for activity factor; average of all trials for both sexes) increase in daily food consumption. In the selected lines, mice with access to running wheels consumed 24.5% more food per day than sedentary indi-

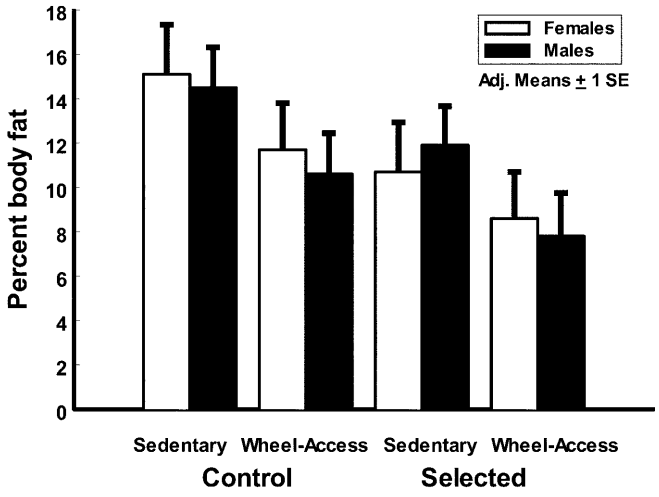

Fig. 3 Percent body fat for female and male mice from the four measurement groups (LSMEANS ± 1 SE, from ANCOVA including sex; Table 1). Lean mass was estimated via hydrogen isotope dilution (Fig. 2) and body fat was calculated as the difference between body mass and estimated lean mass (measured at 105 days of age). Mice with access to freely rotating wheels had less body fat than those housed with locked wheels, and mice from lines selected for high activity had less fat that those from nonselected control lines (see Table 1)

viduals; in the control lines, the difference was 19.5%. Comparable values (13–18% increase) were found in two previous studies of female Swiss albino house mice (Bell and McGill 1991; Bell et al. 1997).

As in a previous study (Koteja et al. 1999b), we found that food consumption adjusted for body mass was positively associated with the number of revolutions run per day (Fig. 4). Not surprisingly, animals that run more expend more energy and therefore require more food. Koteja et al. (1999b) found a higher energetic cost associated with time spent in activity than with distance alone. They concluded that because the selected lines had increased the total movement distance per day (revolutions per day) primarily via increased running speed (rpm), energetic constraints would not likely prevent further increases in daily movement distance.

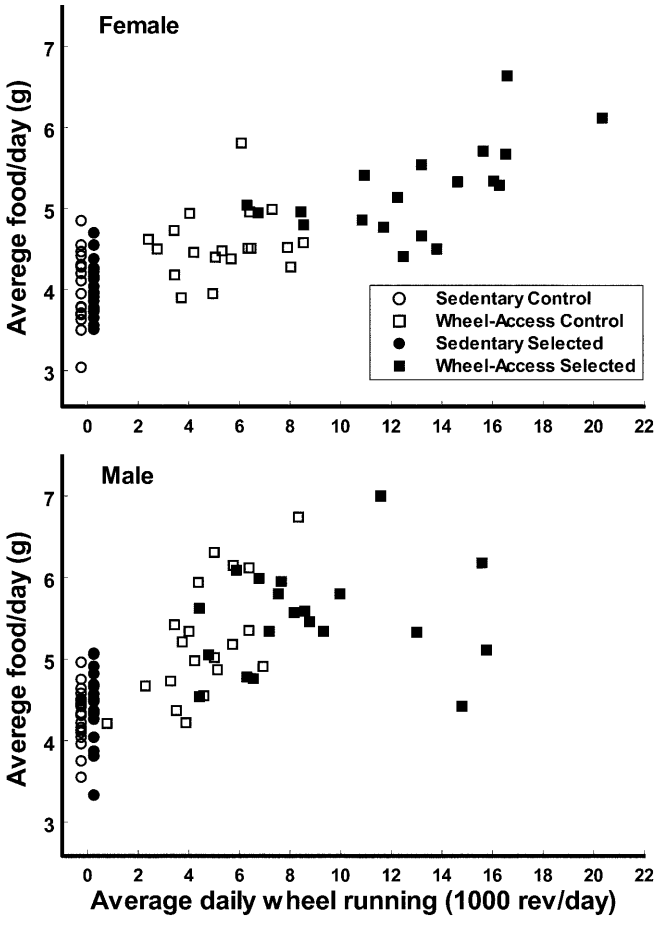

Fig. 4 Food consumption of individual mice in relation to number of revolutions per day of wheel-running activity for females (*top*) and males (*bottom*). Data represent individual variation in average, unadjusted daily values of food consumption and wheel running during the final week of access to running wheels

Based on the fact that we found an energetic cost that appeared to be independent of distance or speed, we suggested the act of remaining in an active state resulted in an additional energy requirement. The present results confirm this hypothesis because mice with access to

Table 1 Results of nested ANCOVA of final body fat (g) of male and female mice (Sex) from selected and control (linetype) housed either with or without access to running wheels (activity). All *P* values are 2-tailed. Tests of hypotheses concerning linetype were performed using Type III MS with Line (linetype) as the error term. Tests of activity and activity×linetype were tested with sex×activity×linetype as the error term. Tests of sex and sex×linetype were tested using sex×line (linetype) as the error term. Tests of sex×activity and sex×activity×linetype were tested using sex×activity×line (linetype)

Source	Body fat (g)				
	df	Type III SS	MS	*F* value	*P*
Sex	1	0.21	0.21	0.18	0.6895
Activity	1	20.79	20.79	5.99	0.0500
Linetype	1	22.44	22.44	7.84	0.0312
Line (linetype)	6	17.18	2.86	2.23	0.0481
Activity×linetype	1	0.67	0.68	0.19	0.6769
Activity×line (linetype)	6	20.84	3.47	2.70	0.0190
Sex×linetype	1	0.00	0.00	0.00	0.9795
Sex×activity	1	0.71	0.71	0.39	0.5553
Sex×activity×linetype	1	0.20	0.20	0.11	0.7524
Sex×line (linetype)	6	7.01	1.17	0.91	0.4921
Sex×activity×line (linetype)	6	10.93	1.82	1.42	0.2172
Time of day	1	3.75	3.75	2.92	0.0914
Time^2	1	9.61	9.61	7.48	0.0076
Age	1	1.23	1.23	0.96	0.3308
Body mass	1	2.07	2.07	1.62	0.2073

functional running wheels consumed 20–25% more food than their counterparts housed with locked wheels, which is much more than predicted by a cost of running revolutions (Fig. 1). However, when comparing selected and control animals that are housed with functional running wheels, differences in food consumption are much smaller (9–13%) and can largely be explained by an incremental cost of running more revolutions (Figs. 1, 4).

In our experiment from generation 10 (Koteja et al. 1999b) when differences in running were 70%, the differences in food consumption (4% difference in mass corrected food consumption) were barely significant in females and actually only approached significance in males. Differences in food consumption between the selected and control lines were only detected for mice housed with access to running wheels. However, as we found a positive association between running and food consumption, we expected that the differences would increase with the further evolution of increased wheel running in the selected lines. Now that the selected lines run more than 2-fold more revolutions per day than controls, we have demonstrated differences in energy intake regardless of housing conditions. Based on our 2-way nested ANCOVA models in the present study, selection resulted in an 8.4% increase in food consumed per day, irrespective of whether mice had access to freely rotating or locked wheels. Dunnington et al. (1981) also found a correlated response in food intake in lines of mice from a short-term (6 generation) selection experiment for high and low wheel-running, even when mice were not housed with access to an activity chamber with a running wheel.

Correlated responses in other behaviors present one possible explanation for the differences in food consumption between the ''sedentary'' selected and control mice; the selected animals could be more active than controls even when housed without access to running wheels, and hence use more energy. Behavioral observations to assess this hypothesis were made during weeks 5–6 of this experiment and were reported in Koteja et al. (1999a). Although we did not observe significant differences between selection groups in locomotor activity outside wheels (in cages) in either ''sedentary'' and ''wheel-access'' groups, we found that the selected mice climbed more in the locked wheels than did the control mice (Koteja et al. 1999a). Thus, even in the ''sedentary'' group, the difference in food consumption rate could still result from a higher energy expenditure for locomotor-related activities in the selected mice. The available data, however, do not allow a quantitative estimation of this additional cost. Another possibility is that the differences in food consumptions resulted from deeper physiological traits, not directly related to behavior. For example, mice in the selected lines could have evolved higher resting metabolic rates. This hypothesis could be tested by direct measurements of basal metabolic rate; such measurements are currently under way.

Body mass

On average, mice from the selected lines were smaller than mice from the control lines before beginning wheel access (Fig. 1, statistically significant for males only). The same relationship is seen at the end of the experiment. The body mass difference between selected and control lines was approximately 6%, and still statistically significant only for males.

In a previous study of generation 14 (Swallow et al. 1999), we reported that mice from the selected lines were significantly lighter (12.9% and 14.3% for females and males, respectively) than their counterparts in the control lines at 79 days of age after 8 weeks of housing individually in locked or freely rotating wheels, beginning at 22 days of age (see also Swallow et al. 1998b). Several protocol differences may explain the smaller relative difference in body mass between selected and control individuals found in the present study. In the present experiment, mice were housed in same-sex groups of up to four individuals from weaning until being given access to running wheels at approximately 7 weeks of age. In our previous experiment, mice were housed individually from 22 days of age. Dunnington et al. (1977, 1981) have shown that genetic correlations between body mass and wheel-running activity change with age, which may explain some of the differences between our two studies. Differences in final body mass revealed by these separate experiments also suggest possible interactions between linetype, social group, age, and growth. The possible mechanism for such an interaction is unknown but might involve suppressed activity, metabolism (but see Speakman and Rossi 1999), thermoregulatory benefits, competition for food, or hormonal changes related to social interactions.

Contrary to a previous study in which wheel-access mice were lighter than their sedentary counterparts (Swallow et al. 1999), final body mass did not differ significantly between ''active'' and ''sedentary'' mice for either sex. The smaller relative effect of access to running wheels found in this study can be explained by protocol differences between the two studies. In the present study, mice were placed on wheels after reaching reproductive maturity, whereas in the previous study mice were placed on wheels at 22 days of age. Chronic exercise regimens initiated before puberty are thought to reduce both lean mass and fat mass, whereas those initiated after puberty are thought to mainly influence fat mass (e.g., Pitts 1984). Furthermore, because all mice (including sedentary individuals) were given access to running wheels for 6 days at the beginning of the present experiment, the effect of access to running wheels may have been obscured. Body mass declines somewhat before beginning to increase in all groups (Fig. 1).

Body composition

Lean body mass of mice from the selected lines did not differ significantly from lean body mass of mice from the

control lines for either sex. Similarly, lean body mass did not differ between mice housed with or without access to functional wheels. We did, however, find that both access to running wheels and previous artificial selection for high activity resulted in reduced fat accumulation (Fig. 3). Thus, for this set of experimental conditions, a change in fat mass is sufficient to explain the small differences in body size between selected and control lines. These results suggest that factors which influence body size in the selected lines are acting through an intermediate phenotype of activity rather than through interactions with fundamental mechanisms underlying variation in growth rate per se.

Although we found apparently significant differences between males and females in total body fat, this is largely a function of body mass, as models of fat that include body mass as a covariate, or in which fat is expressed as percent body fat, do not indicate a significant effect of sex (Table 1). Nor did we find a significant interaction between sex and either linetype or activity group. These results contradict the hypothesis that females are better able to protect fat reserves as an adaptation to the increased energetic demands of parental care, a cost disproportionately born by females in mammals (e.g., Hoyenga and Hoyenga 1982; Perrigo and Bronson 1985). In contrast, studies of laboratory rats given exercise have been cited in support of this hypothesis because significant sex differences in fat deposition and usage have been found under both forced (Nance et al. 1977; Applegate et al. 1982) and voluntary (Pitts 1984; Cortright et al. 1997) exercise regimens. Rats do not present the best model to test the adaptive parental care hypothesis, however, because differences in body composition could also be attributed to sexual differences in appetite when challenged with exercise. Male rats with wheel access or under forced exercise either do not increase food consumption or increase it only marginally compared with female rats (e.g., Pitts 1984). We show that both male and female mice compensate for increased energy demand of voluntary exercise by increasing food consumption (see also Bell and McGill 1992; Bell et al. 1997). We also found no difference in fat deposition between the sexes and, thereby, no support for the idea that females protect fat reserves more competently than males (Fig. 3; Table 1).

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