

Energy Cost of Wheel Running in House Mice: Implications for Coadaptation of Locomotion and Energy Budgets

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ABSTRACT

Laboratory house mice (*Mus domesticus*) that had experienced 10 generations of artificial selection for high levels of voluntary wheel running ran about 70% more total revolutions per day than did mice from random-bred control lines. The difference resulted primarily from increased average velocities rather than from increased time spent running. Within all eight lines (four selected, four control), females ran more than males. Average daily running distances ranged from 4.4 km in control males to 11.6 km in selected females. Whole-animal food consumption was statistically indistinguishable in the selected and control lines. However, mice from selected lines averaged approximately 10% smaller in body mass, and mass-adjusted food consumption was 4% higher in selected lines than in controls. The incremental cost of locomotion (grams food/revolution), computed as the partial regression slope of food consumption on revolutions run per day, did not differ between selected and control mice. On a 24-h basis, the total incremental cost of running (covering a distance) amounted to only 4.4% of food consumption in the control lines and 7.5% in the selected ones. However, the daily incremental cost of time active is higher (15.4% and 13.1% of total food consumption in selected and control lines, respectively). If wheel running in the selected lines continues to increase mainly by increases in velocity, then constraints related to energy acquisition are unlikely to be an important factor limiting further selective gain. More generally,

our results suggest that, in small mammals, a substantial evolutionary increase in daily movement distances can be achieved by increasing running speed, without remarkable increases in total energy expenditure.

Introduction

Locomotion has received attention from ecological and evolutionary physiologists for various reasons. First, locomotor performance abilities (e.g., speed, stamina) may generally be related to survival chances of prey and foraging success of predators. At the level of individual variation within populations, some studies have shown or suggested that natural selection does indeed favor individuals with high performance abilities (e.g., Christian and Tracy 1981; Jayne and Bennett 1990; D. B. Miles, personal communication; but see Brodie 1992). Among species, a few studies have shown relationships between locomotor performance abilities and behavior or ecology (e.g., Hertz et al. 1988; Losos 1990; Garland and Losos 1994).

Second, costs of locomotion are incorporated, explicitly or implicitly, in various models of resource acquisition, territory defense, or migratory behavior (see reviews: Schoener 1987; Alerstam 1991). Because energy expenditure during locomotion can be very high relative to expenditure at rest, optimal foraging strategies may depend strongly on the energetic cost of locomotion per unit distance moved or per unit time active (Reichman 1981; Janetos 1982; Speakman 1986). In mammals, for example, rates of energy metabolism measured during locomotion may exceed resting values by a factor of seven to more than 30 (Taylor et al. 1981; Koteja 1987; Lindstedt et al. 1991). Moreover, distances traveled in nature can be great, even in small mammals (Garland 1983; Goszczynski 1986). Therefore, locomotor costs might be an important part of the total daily energy budget.

However, calculations based on allometric equations for distances covered by free-living animals, the costs of running measured on laboratory treadmills, and total daily energy expenditure indicate that locomotor costs can actually be a small part of an animal's total energy budget, especially for small mammals (Garland 1983; Altman 1987; Baudinette 1991). For a mouse-sized mammal, the estimate may be as low as 1%. The estimate increases with body size, but even for large carnivores, which have the highest daily movement distances, it is generally

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below 15% of the total energy budget (Garland 1983; Goszczynski 1986; but see Gorman et al. 1998).

Several workers have argued that costs of locomotion in nature can be much higher than calculated in the above way because of underestimated daily movement distances and/or because the cost of running on treadmills may be lower than for running on natural substrates (Altman 1987; Karasov 1992). Direct estimates of the proportion of locomotion costs in energy budgets in free-living animals require simultaneous measurements of metabolic rates and observations of behavior. This approach, applying the doubly labeled water method, proved successful in estimating costs of flight in birds (Masman and Klaassen 1987). Small mammals are usually more difficult to observe in the wild, however, and studies are scarce.

In the most detailed study of a small mammal (the ground squirrel *Spermophilus saturatus*) performed to date, Kenagy and Hoyt (1989) combined laboratory measurements of oxygen consumption as animals ran on a treadmill (Hoyt and Kenagy 1988) with field measurements of energy expenditure, running speed and distance, and time budgets. The estimated net costs of locomotion amounted to 13% of total daily energy expenditure. This value is more than 10 times higher than that predicted by equations in Garland (1983) or Altman (1987) and is comparable to other major components of energy budgets of the ground squirrels, such as thermoregulation or reproduction (Kenagy and Hoyt 1989). In other studies using similar approaches, the estimated cost of locomotion amounted to 2%–4% of daily energy expenditure (Karasov 1992).

The magnitude of locomotor costs has important ecological and evolutionary implications. If locomotor costs are high, then we might expect the evolution of longer daily movement distances to be accompanied by the evolution of various morphological and physiological specializations that would reduce the energetic cost of locomotion. Species of lizards (Autumn et al. 1997) and of mammals (Taylor et al. 1982) have been shown to differ substantially in the cost of locomotion, measured per unit distance or per unit time, but the mechanistic bases of this variation are as yet unclear (e.g., Farley and McMahon 1992; Steudel and Beattie 1995; Meyers and Steudel 1997; Roberts et al. 1997). Moreover, no study has yet shown that interspecific variation in the cost or efficiency of locomotion actually relates to variation in daily movement distance, home range area, or any other behavioral or ecological variable. Thus, the importance of locomotor cost or efficiency during correlated evolution of morpho-physiological and behavioral traits is not obvious (Garland 1983; but see Autumn et al. 1997).

An experimental evolutionary approach can be used to circumvent the logistical and practical difficulties of comparative and field studies of wild animals. Specifically, it is possible, in principle, to use selective breeding to change either locomotor costs or efficiency or to change daily movement distances. As one trait evolves, other traits can be monitored for correlated responses. We have taken this approach with laboratory house

mice (*Mus domesticus*) as a model system. Beginning from a base population of outbred Hsd:ICR mice (Hauschka and Mirand 1973; Dohm et al. 1996), we have selected for increased levels of voluntary activity on running wheels (Swallow et al. 1998a) and tested for correlated responses in various morphological and physiological traits (Swallow et al. 1998b).

In the present study, we focus on the effects of increased activity on food consumption and energy budgets of the mice. We hypothesized that mice from selected lines would have, on average, higher daily energy expenditures than mice from control lines. We also test whether the amount of running is positively correlated with energy expenditures at the level of individual variation. We point out as well that the evolution of higher overall activity levels might be accomplished by increases in the amount of time spent active and/or the intensity (velocity) of activity and discuss the energetic and evolutionary implications of these different possibilities.

Material and Methods

Animals

Outbred, genetically variable Hsd:ICR mice (Hauschka and Mirand 1973; Dohm et al. 1996; Carter et al. 1999) were used to establish eight lines in 1993 (Swallow et al. 1998a). In four of the lines, mice have been selected for high voluntary wheel running (measured at the age of 35 to 55 d as a total number of wheel revolutions per day); the other four have been randomly bred. See Swallow et al. (1998a) for details of animal maintenance and selection protocol. The current experiment was performed on 148 individuals of generation 10 postselection (from second litters). The mice were housed individually from weaning in cages equipped with wheels (circumference = 1.12 m). The experiment began when the mice were 64–80 (average 76) d old.

Protocol

Rates of food consumption and energy assimilation were measured in 3-d trials in plastic mouse cages (27 × 17 × 12.5 cm) with perforated polypropylene plates suspended over the floor (Koteja 1996). No bedding or nesting material was provided. The grids were placed in the cages 10 d before the first of the feeding trials. The first trial was performed in the cages with wheels attached to their side, accessible through an opening in the wall of the cage. The number of wheel revolutions was monitored with 1-min resolution. The raw readings were used to calculate total number of revolutions per day, number of 1-min intervals during which any wheel running occurred, and an average number of revolutions per interval with any running (an estimate of average running speed). In further analyses, we used average values of the second and third day of the trial because wheel running during the first day was apparently affected by the initial manipulation (see Mather 1981). The next

three trials were performed in similar cages, but without attached wheels. The trials were performed consecutively, so that the end of a trial was the start of the next. The measurements were performed simultaneously in all animals.

Food Consumption and Energy Assimilation

At the beginning of each trial, animals were weighed (± 0.1 g), and a weighed portion of food (± 0.01 g, Harlan Teklad Rodent Diet [W] 8604) was placed on the grids. Water was provided ad lib. Samples of the food were taken to measure dry mass content. After each trial the cages were changed. All uneaten food (including orts) and feces were collected, segregated manually, and dried at 60°C to constant mass. Energy content of dry food (E_{food} ; 17.73 kJ/g) and dry feces (E_{feces} ; 14.93 kJ/g) was measured with a Phillipson Microbomb Calorimeter (Gentry Instruments). We sampled the feces from one male and one female from each line; because no significant differences between sexes or selection groups were detected, we used an average value in further calculations.

Food consumption rate (C , g/d) was calculated as:

$$C = (\text{food given} \\ \times \text{dry mass content}) - (\text{dry food uneaten}).$$

In the trials without the wheels, apparent digestibility of dry mass (d_m) and energy (d_e) were calculated as:

$$d_m = (C - \text{feces mass})/C \\ d_e = [(C \times E_{\text{food}}) - (\text{feces mass} \times E_{\text{feces}})]/(C \times E_{\text{food}}),$$

and the rate of energy assimilation was calculated as:

$$A = [(C \times E_{\text{food}}) - (\text{feces mass} \times E_{\text{feces}})] \times 0.97.$$

The value is multiplied by 0.97 to account for an assumed 3% energy loss in urine (Drozdz 1975; Grodzinski and Wunder 1975).

During the trials with wheel access, some amount of feces was lost from the collecting trays as the animals ran on the wheels. Therefore, to avoid overestimating the rate of energy assimilation, we used individuals' average digestibility coefficients measured in the three trials without wheels (d_e) to calculate assimilation rate in the trial with wheel access:

$$A_{\text{on wheels}} = C_{\text{on wheels}} \times d_e \times 0.97.$$

The rates of energy assimilation calculated in this way were on average 2% lower than calculated with the previous equation.

Statistical Analysis

Two-level-cross-nested ANOVA was used to test simultaneously the effects of selection, variation among the replicate lines within the selection and control groups, random variation among families within lines, and sex. To test statistical significance of the effects, the appropriate F -values were calculated as follows (Sokal and Rohlf 1981; Henderson 1989): effect of selection over variation among lines, random effect of replicate lines over variation among families, effects of sex and sex \times selection interaction over sex \times lines interaction term. Because only one male and one female per family were used, sex \times family interaction could not be tested, and it was not included in the model. The effects of family and sex \times line interaction were tested over the residual (error) variance, which in this model was equal to the variance associated with sex \times family interaction term. Dependent variables were body mass, body mass changes, digestibility coefficients, number of wheel revolutions per day, number of 1-min intervals during which any wheel activity occurred, and average running speed (total number of wheel revolutions divided by the number of intervals with any revolutions). An inverse measure of wheel resistance, measured as a number of free wheel rotations after spinning the wheels to a constant speed, was included as a covariate when comparing wheel activity. Differences in food consumption and energy assimilation rates were tested with similar ANCOVA models, with body mass and body mass changes as covariates. Before estimating the final ANCOVA models, homogeneity of slopes was tested with models including appropriate interaction terms. Finally, the amount of wheel activity was also included as a covariate, to evaluate the relation between food consumption and activity at the level of individual variation.

Because males are much larger than females, results of ANCOVA with body mass as a covariate are very sensitive to the assumption of a common regression slope, and the tests of the effect of sex are suspect. Therefore, simpler ANCOVA models for separate sexes were also estimated. The models included only one fixed factor (selection) and one nested random effect (lines within selection groups), plus covariates (age, in addition to those used in the previously described models) and a block variable (housing room number). Family does not appear in the model because each individual represented a different family. In a few families, only one individual was available; these data were omitted in the analyses including sex and family effects but were included in the simpler models for separate sexes.

We hypothesized that mice from the selected lines would run more revolutions per day, spend more time running, run at higher average velocities, and have higher rates of food consumption and of energy assimilation. We also expected a positive relation between the consumption or assimilation rates and body mass, wheel-running activity, and body mass changes.

To test those a priori hypotheses, one-tailed tests are appropriate; therefore, significance levels were calculated by halving the usual P -values (Sokal and Rohlf 1981, p. 226).

Preliminary analysis indicated that the distribution of body mass, total revolutions, average running speed, and wheel resistance, were significantly right skewed. Therefore, the final analysis and significance testing were performed with transformed variables: $\log_{10}(\text{body mass})$, $(\text{total revolutions})^{0.5}$, $(\text{average speed})^{0.5}$, $(\text{wheel resistance})^{0.5}$. A few individuals appeared as severe outliers with respect to running activity, food consumption, or changes of body mass. We assumed that those were results of a mistake in weighing the food, a malfunction of the wheel recorder or on obstructed water bottle leading to large body mass decline. The records were excluded from analysis. All statistical analyses were performed with SYSTAT 6.0 for Windows (SPSS).

Estimating the Cost of Wheel-Running Activity

The cost of running was estimated as a partial regression slope coefficient in an ANCOVA model with food consumption (or energy assimilation) as the dependent variable and the amount of wheel activity as a covariate. Other variables included in the model were body mass and body mass change and group categories (selection, line, sex, family).

The amount of wheel activity was included in the models as the number of wheel revolutions (in 1,000/d) or as the number of 1-min intervals with any wheel activity (intervals/d). In the first case, the coefficient measures the incremental cost of running (covering distance; g food/1,000 revolutions or kJ/1,000 revolutions), in the latter it measures the cost of time active (g food/interval or kJ/interval). Both with revolutions and with intervals, the amount of activity was included either as a stand-alone covariate or as an interaction with body mass. In the first case, the coefficient measures the cost per animal; in the latter case, it measures a mass-specific cost of wheel activity (per gram of body mass).

Results

Wheel-Running Activity

The number of wheel revolutions per day varied among individuals from about 1,000 to 19,000 (Fig. 1). On average, mice from selected lines ran about 70% more than the mice from control lines, and, within all lines, females ran more than males (Table 1). The analysis also revealed significant variation among the lines within selection groups and among families within the lines. The analysis performed for separate sexes indicated that in males the amount of running was negatively correlated with body mass ($P = 0.028$). In females, the correlation was positive but not significant ($P = 0.231$; Fig. 1). In the model

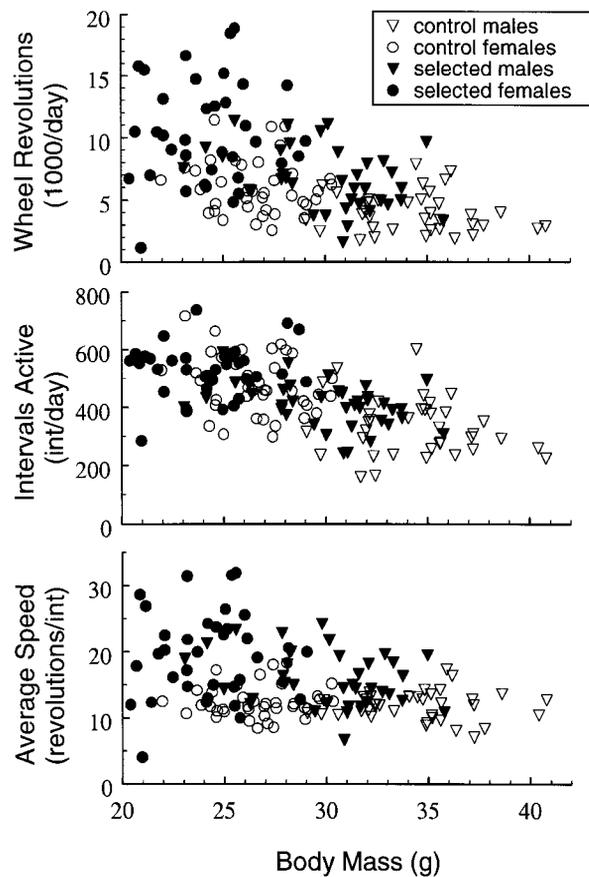


Figure 1. Wheel activity measured as number of wheel revolutions (*top*), number of 1-min intervals during which any revolutions occurred (*middle*), and average number of revolutions per active interval (*bottom*) in relation to body mass in laboratory house mice.

including body mass as a covariate, the effect of selection was less profound in males ($P = 0.046$; selected/control ratio of back-transformed adjusted means = 1.43), but in females, the effect was as high as in the model without body mass ($P = 0.015$; selected/control ratio = 1.80).

The number of 1-min intervals with wheel activity ranged from 160 to 740 per day (Fig. 1). On average, it was 36% higher in females than in males, but only 17% higher in mice from the selected lines than in those from control lines (Table 1). Variation among lines and among families was also significant. When the sexes were analyzed separately, the effect of selection was significant only in males. Body mass did not affect the trait significantly (females: $P = 0.417$; males: $P = 0.091$).

Average number of revolutions per 1-min active interval varied among individuals from 4 to 32 (Fig. 1). Mice from the selected lines ran 46% faster than those from the control lines (Table 1). In control lines, the difference between sexes in run-

Table 1: Body mass and running activity in laboratory house mice: significance of the main effects tested by ANOVA or ANCOVA

Trait/Sex	Average \pm SD				Significance of Effects (P -values from ANCOVA) ^a					
	Selected		Control	Ratio	Selected	Replicate Lines	Sex	Family	Sex \times Selected	Sex \times Line
Initial body mass (g) ^b		020	.003	.000	.000	.268	.004
Females	24.3 \pm 2.3	26.7 \pm 2.191	.051	.001
Males	30.1 \pm 3.0	34.4 \pm 2.888	.006	.033
Revolutions (1,000/d)		008	.000	.000	.046	.247	.347
Females	10.4 \pm 4.1	6.0 \pm 2.2	...	1.73	.011	.003
Males	6.6 \pm 2.5	4.0 \pm 1.6	...	1.68	.006	.016
Intervals active (1/d)		031	.025	.000	.026	.768	.105
Females	527 \pm 90	476 \pm 101	...	1.11	.119	.008
Males	407 \pm 77	331 \pm 98	...	1.23	.023	.061
Average speed (rev/int)		007	.001	.012	.015	.067	.655
Females	19.3 \pm 6.6	12.3 \pm 2.4	...	1.57	.006	.008
Males	15.9 \pm 4.1	11.8 \pm 2.2	...	1.35	.005	.049

Note. The first line for each trial shows the results for both sexes, the latter two show the results for separate sexes. Number of individuals: 37 selected males, 38 control males; 37 selected females, 37 control females.

^a $P = 0.000$ indicates $P < 0.0005$.

^b Measured before start of first trial (with wheels).

ning speed was small, but in the selected lines, females ran 21% faster than males. The average speed decreased with body mass in males ($P = 0.045$); in females the trend was positive but not significant ($P = 0.260$).

Body Mass and Body Mass Changes

Initial body mass of the mice ranged from 20.4 to 40.8 g (Fig. 1). Males were larger than females, and mice from control lines were larger than those from the selected lines (Table 1; Fig. 2). Significant variation among lines within the selected and control groups, and among families within lines, was also detected. A significant interaction between the effects of sex and line (Table 1) indicated that the differences among the replicate lines depended on sex. Body mass of females was slightly affected by their age ($P = 0.051$), but in males the effect was absent ($P = 0.455$).

Body mass changes were, on average, only slightly negative in the trial with the wheels, and none of the effects tested (selection, lines, sex, families) were significant (Fig. 2). However, in the first trial without wheels, mice from selected lines had negative body mass changes, while control mice maintained almost constant body mass (effect of selection: $P = 0.016$). In the next trial, the trend was similar (positive changes in the control lines but negative in the selected ones), but the effect was not significant. In the final trial, body mass changes were positive in both groups; differences among the lines, families, or sexes were not statistically significant (Fig. 2).

Digestibility and Energy Assimilation

In the three trials without wheels (trials 2–4), the coefficients of dry mass digestibility (apparent) averaged 74.9%, 73.9%, and 74.7%, respectively. Corresponding values for energy digestibility were 78.8%, 78.1%, and 78.7%. Although the differences among the trials were very small, repeated measures ANOVA indicated that the values in trial 3 were significantly lower. None of the effects tested (selection, sex, lines, families) significantly affected the digestibility coefficients, except a significant variation among the lines in trial 3 only ($P = 0.003$). However, the estimates of digestibility were repeatable across the trials at the level of individual variation (correlations between the individuals' values obtained in trials 2 and 3 [$r = 0.41$], trials 2 and 4 [$r = 0.43$], and trials 3 and 4 [$r = 0.37$] were all statistically significant; pairwise $P < 0.0001$). Therefore, for each individual, an average of the three trials was used as an estimate of digestibility coefficient, to be used in calculations of the rate of energy assimilation in the trial with wheel access.

Because digestibility coefficients were not affected by either selection or sex, and the differences among the trials were small, the results for energy assimilation were qualitatively similar to those for the rates of food consumption. Therefore, only the latter will be presented in detail in the next section.

Food Consumption Rate

As hypothesized, food consumption (raw data) was highest in the trial with wheel access (4.88 g/d) and decreased in the three

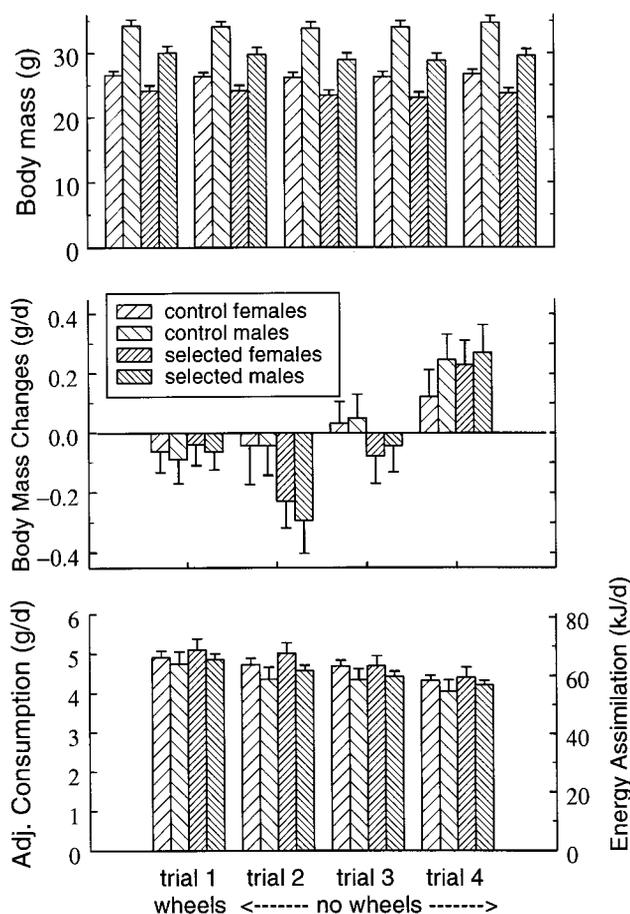


Figure 2. Average body mass (measured between trials; *top*), body mass changes during the trials (*middle*), and adjusted food-consumption rates (*bottom*) in a trial with wheel access (trial 1) and subsequent trials without wheels (trials 2–4). The values are given with ± 2 SE. Food consumption rates are adjusted least square means from ANCOVA (separate for each trial) including the effect of body mass, body mass changes, selection, line, family, and sex. The adjusted means were always higher in females. However, as explained in the text, the comparison between sexes should be treated with caution. Energy assimilation rate (right Y-axis) is an approximation, based on overall average energy assimilation coefficient (see Table 2 for exact values). Difference between selected and control lines is statistically significant only for females in trial 1.

following trials without wheels (4.68, 4.53, and 4.24 g/d; $P < 0.001$, both for an overall repeated measures ANOVA and planned contrasts between the trials; see also Fig. 2).

As expected, food consumption was positively correlated with body mass (Table 2; Fig. 3). In the trial with wheel access, food consumption was positively related to body mass changes in females ($P = 0.031$) but not in males. ANCOVA adjusted least square means tended to be higher in females than in males, but the effect was not significant (Fig. 2).

Because of the large difference in body size, the comparison

between sexes was suspect. A preliminary analysis also indicated a possible second-order interaction of body mass, sex, and selection ($P = 0.075$), and an interaction between body mass changes and sex ($P = 0.060$). When the sexes were analyzed separately (Table 2), the relation between food consumption rate and body mass was stronger, and the common regression slopes were steeper than in the ANCOVA for both sexes.

Whole-animal food consumption did not differ between selected and control mice (left columns of Table 2). However, food consumption adjusted for effects of body mass and body mass changes (ANCOVA) was higher in the selected lines, although the difference was statistically significant only in females (Table 2). Variation among replicate lines was significant in males but not in females.

ANCOVA with number of wheel revolutions or number of active intervals as a covariate showed that both significantly affected food consumption (revolutions^{0.5}: males $P = 0.002$, females $P = 0.029$; intervals: males $P = 0.001$, females $P = 0.003$; Fig. 4). However, the covariates did not explain all the differences among the lines. In males, the variation among lines remained significant after including the number of revolutions ($P = 0.009$) or the number of running intervals as a covariate ($P = 0.017$). In females, the effect of selection was not significant with the number of revolutions as a covariate ($P = 0.274$) but remained significant with number of active intervals as a covariate ($P = 0.042$).

In the three trials without wheel access, food consumption rate was positively related to body mass changes in both sexes ($P < 0.001$). The partial regression coefficient ranged from 0.85 to 1.10 g food consumed per gram body mass change (or 12.8 to 16.6 kJ assimilated per gram mass change). Consumption adjusted for those effects was always higher in females than in males, but the effect was significant only during the first of the trials ($P = 0.015$; Fig. 2). In all the trials, consumption (adjusted for the effects of body mass and body mass changes) in the selected lines was higher than in the control lines, but the difference was small and not statistically significant (Fig 2).

Cost of Wheel-Running Activity

The cost of activity was estimated as a partial regression slope in a model with food consumption (or energy assimilation) as the dependent variable, and the amount of activity as one of the covariates (Table 3). The cost of activity measured per revolution tended to be higher in males than in females. This difference did not seem to be a result of a difference in body mass, because the mass-specific cost of locomotion also tended to be higher in males than in females (Table 3, right column). However, in the model with both sexes, the activity \times sex interaction term was not significant, which suggests that the apparent difference in the cost of running between sexes might not be real. The selection \times revolutions or selection \times active-intervals interactions were not significant in any of the

Table 2: Food consumption and energy assimilation in the trial with wheel access

	Raw Data Average (\pm SD)		Adjusted (ANCOVA) Means (\pm SE) ^a			Significance of Effects (<i>P</i>) ^b			
	Selected	Control	Selected	Control	Ratio	Selected	Replicate Lines	Body Mass	Mass Change
Food consumption (g/d):									
Females	4.80 \pm .45	4.76 \pm .37	4.91 \pm .07	4.66 \pm .07	1.05	.041	.238	.001	.031
Males	4.93 \pm .42	5.07 \pm .41	5.09 \pm .06	4.94 \pm .06	1.03	.238	.001	.000	.240 ^c
Energy assimilation (kJ/d):									
Females	64.6 \pm 6.0	64.3 \pm 5.1	66.0 \pm .89	63.0 \pm .87	1.05	.060	.169	.000	.023
Males	66.6 \pm 5.8	68.7 \pm 5.3	68.6 \pm .80	67.0 \pm .80	1.02	.300	.000	.000	.122 ^c

^a The adjusted means in the table are from ANCOVA for separate sexes, and cannot be used to compare the sexes. See Table 1 for number of individuals tested and body mass data.

^b *P* = 0.000 indicates *P* < 0.0005.

^c The slope of the relation between consumption and body mass change in males was, unexpectedly, negative. The *P*-value is for two-tailed test.

models examined. This indicates that there were no detectable differences in the cost of wheel-running activity between the selected and control lines.

The number of wheel revolutions or active intervals (from Table 1) multiplied by the respective cost coefficient (Table 3) provided an estimate of the additional daily food consumption associated with wheel activity. The daily cost of covering distance (revolutions) amounted to about 0.21 g dry food per day in control mice and 0.37 g in selected mice. These values are only about 4.4% of the total food consumption (from Table 2) in the control lines and 7.5% in the selected ones. The daily cost of time active (number of active intervals from Table 1 multiplied by cost coefficient from Table 3) was considerably higher: 0.64 g of additional food per day (13.1% of energy budget) in the control lines and 0.75 g (15.4% of energy budget) in the selected lines (see also Fig. 5).

If the cost of wheel activity is a simple additive component of the energy budgets, then food consumption in the trial with wheels, reduced by the cost of activity, should equal food consumption in the following trials without wheels. A direct comparison could not be done, however, because the mice did not maintain a constant body mass—which also affected the rate of food consumption (see above). Moreover, the pattern of body mass changes differed between sexes and selection groups (Fig. 2). Therefore, we calculated food consumption adjusted to zero body mass changes (ANCOVA) separately for the four sex/selection groups (Fig. 5). The adjusted food consumption, which can be treated as a measure of daily energy expenditure, decreased in consecutive trials without wheels to values lower than predicted by subtracting the cost of covering distance on the wheels (hatched bar in Fig. 5). Note, however, that the change was not immediate; the rate of energy expenditure during the first trial without wheels was as high as in the trial with wheels. In the last trial, the adjusted food consumption was

very close to the values predicted by subtracting the cost of time of wheel activity (crossed bar on Fig. 5).

Discussion

Cost of Wheel Running

Average daily distances traveled by the mice studied herein, estimated as a product of the number of revolutions and wheel circumference (1.12 m), ranged from 4.4 km in the control males to almost 11.6 km in selected females. This is an order of magnitude more than the daily movement distance predicted for a free-living, mouse-sized mammal (allometric equations in Garland 1983). Wheel measures are biased upward because the mice spent some time coasting in the wheels and because the wheels can run freely for some time after a mouse has exited. It also seems likely that the energetic cost of running in a wheel is lower than in a natural environment with uneven substrate. Nevertheless, the daily locomotor effort in the mice caged with wheels may be at least comparable to that of free-living animals.

Our results demonstrate that the incremental cost of wheel-running represents only a small portion of the total energy budgets of the mice. An average cost of 1,000 wheel revolutions (about 1.12 km) was 0.89 kJ of energy assimilated in males and 0.38 kJ in females. Total incremental cost of running amounted to 4.4% of energy budgets in the mice from control lines and 7.5% in the mice from the selected lines. However, we should make clear precisely what kind of information these results provide.

The cost coefficients estimated in this study measure an additional amount of energy assimilated (or food consumed) associated with a certain behavior, rather than an energy cost of running per se. It is a trivial assertion that running requires more energy than resting and that, other things being equal,

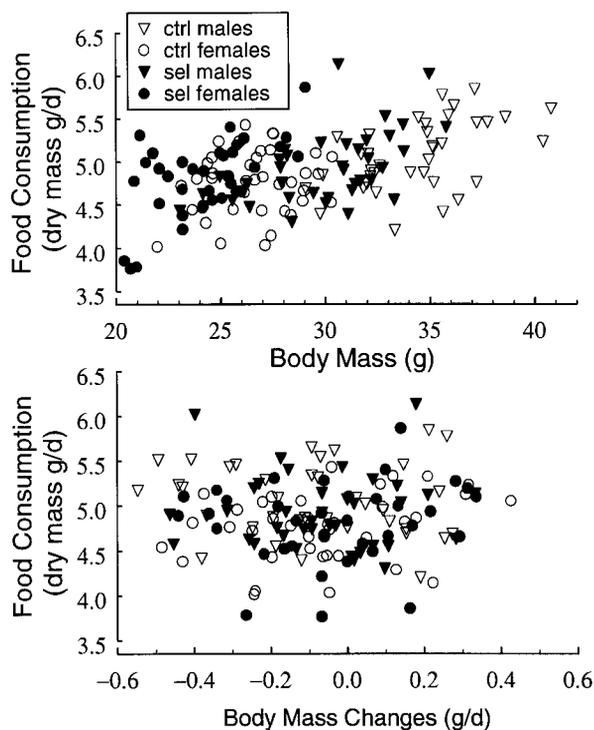


Figure 3. Food consumption rate of individual mice in relation to body mass (*top*) and body mass changes (*bottom*), during trial with wheel access.

animals that run more spend more energy for running. However, this does not imply that the total energy expenditure of an animal that runs more must be higher. For example, wheel running might be negatively correlated with other energy-demanding behavioral traits, such as nest building (Lynch 1994). In fact, when given access to running wheels, mice from our selected lines do build smaller nests when provided with cotton (which was not available in the present experiments) (P. A. Carter et al., unpublished results; see also Bult et al. 1993).

Thus, high-running individuals might not necessarily have higher daily expenditures. Our demonstration of a positive relationship between the amount of voluntary running and the body mass-adjusted food consumption (or energy assimilation rate) is, to our knowledge, the first study to clearly demonstrate such an effect at the level of individual variation. Some previous studies have indicated that rats and mice with access to wheels had higher food consumption than their sedentary counterparts (Holloszy 1993; MacNeil and Hoffman-Goetz 1993), but the actual cost of the wheel-running activity has not been estimated.

In the trials with wheel access, mass-adjusted consumption and assimilation were 2%–5% higher in the selected lines than in the control lines, although the difference approached significance level only in females (Table 2). However, the difference between the selected and control lines is just as expected from

the estimates of cost of activity and the difference in the number of wheel revolutions (3.3%; Fig. 5). The agreement between the two values is nontrivial because the estimates of the cost coefficients were derived from ANCOVA models that controlled for the effect of selection. Because the test of significance for the effect of selection was based on only six degrees of freedom (variation among the eight replicate lines nested within selection and control groups), the chances of statistically rejecting a null hypothesis were low for such a small effect.

It is of interest to compare the above estimates of the cost of running with estimates of the incremental cost of locomotion of animals running on treadmills. Such estimates are generally taken as the slope of the regression of sustained aerobic metabolic rate on treadmill speed. At present, we do not have such data for our mice. As an approximation, we can use a prediction from an equation relating the incremental cost of terrestrial locomotion to body mass (M ; Taylor et al. 1982): incremental cost of locomotion (kJ/km) = $10.7 M(\text{kg})^{0.684}$. The predicted incremental cost of locomotion is 1.02 kJ/km for males (32.2 g), and 0.87 kJ/km for females (25.5 g) used in this study. Three studies in which the measurements were performed on house mice yield similar values: 1.19 kJ/km (body mass = 21 g), 0.87 kJ/km (30 g), and 0.69 kJ/km (33 g) (from table 1 of Taylor et al. 1982). The values are close to our estimate

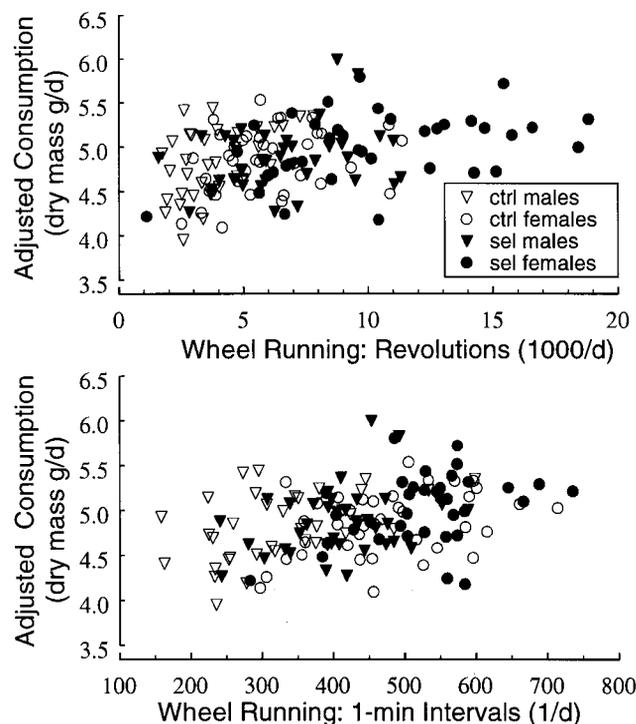


Figure 4. Food consumption rate of individual mice adjusted for the effects of body mass and body mass changes in relation to number of wheel revolutions (*top*) and number of active intervals (*bottom*).

Table 3: Estimates of the cost of wheel-running activity in laboratory house mice, calculated as the slopes in ANCOVA models with food consumption or energy assimilation as dependent variables, and amount of wheel activity as one of the independent variables

	Per Animal		Per Gram of Body Mass	
	Effect	<i>P</i>	Effect	<i>P</i>
Food consumption:				
g/1,000 revolutions:				
Males064	.002	.0020	.003
Females030	.029	.0011	.039
Both038	.024	.0014	.024
g/1-min intervals:				
Males0018	.001	.000055	.000
Females0015	.003	.000055	.004
Both0017	.003	.000055	.004
Energy assimilation:				
kJ/1,000 revolutions:				
Males89	.002	.028	.002
Females38	.035	.014	.045
Both45	.030	.018	.028
kJ/1-min interval:				
Males024	.000	.00076	.000
Females019	.004	.00070	.005
Both021	.004	.00071	.005

Note. Significance of the effect; $P = 0.000$ indicates $P < 0.0005$.

based on energy assimilation and number of wheel revolutions in males (1,000 revolutions = 1.12 km; 0.89 kJ/1,000 revolutions = 1.00 kJ/km). This result suggests that the cost of running directly translates into increased energy expenditure in males. In females, however, the cost predicted from the treadmill measurements is twice that measured in our study (0.38 kJ/1,000 revolutions = 0.43 kJ/km). At least two hypotheses can be proposed to explain this discrepancy. First, females may indeed be more economic runners than males. Second, and more probably, females and males may differ behaviorally in ways that bias the relative cost estimates. For example, females might coast on the running wheels more than males do, so that the distance females run is substantially overestimated. Alternatively, individual females that run more on wheels might be less active in other ways, which, across individuals, would reduce the slope of food consumption regressed on wheel revolutions. The first hypothesis could be tested by direct measurements of oxygen consumption in running animals, the second by direct observations of behavior.

Distance Traveled versus Time Active

The cost of time of the wheel-running activity (measured as additional food consumption or energy assimilation per active

interval; Table 3), multiplied by number of active intervals (Table 1) provides an estimate of daily cost of time active. The additional energy amounted to 12%–16% of the total energy budget of the mice, more than twice as much as the cost of running revolutions (see also Fig. 5). At first glance, the result seems paradoxical because both costs are based on the same activity. The difference, however, can be explained and also allows new insights.

The cost of running measured as revolutions per day estimates only an incremental cost of locomotion, related to distance traveled. As indicated above, it is comparable to incremental cost of locomotion estimated for animals running on treadmills. However, the total cost of locomotion also includes a component independent of running speed (which can be estimated as the Y-intercept of the regression of metabolic rate on running speed). Thus, energy expenditure of a resting animal can be much lower than that of an active one, even when the activity level is low (Fig. 6). A simple model (Fig. 6) based

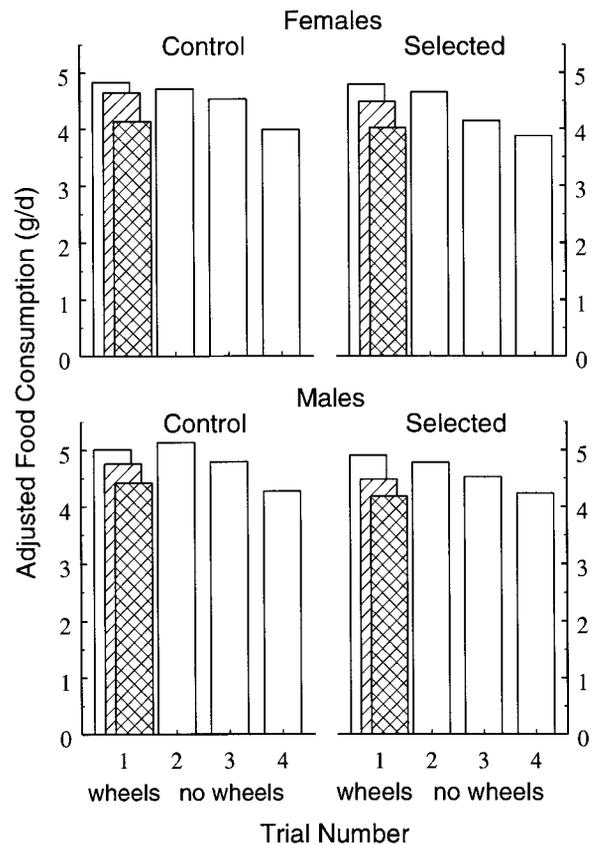


Figure 5. Daily food consumption adjusted for zero body-mass changes (open bars) in the trial with wheels (trial 1) and in the following trials without wheel access (trials 2–4). In trial 1, additional bars indicate food consumption reduced by the estimated daily incremental cost of running (wheel revolutions; hatched) or by daily incremental cost of time active (intervals with activity; crossed).

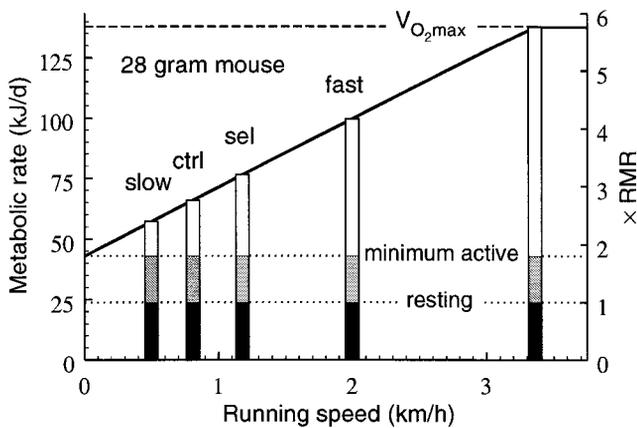


Figure 6. A model of cost of locomotion in laboratory house mice. The solid line describing a relation between running speed (on treadmill) and metabolic rate is an average of predictions from three regression lines for house mice (average body mass 28 g) from Taylor et al. (1982; Table 1). Resting metabolic rate (RMR) is an average of values given by Taylor et al. (1970) and Meyer and Guillot (1986), scaled for a 28-g mouse (assuming 0.7 exponent of the relation between metabolic rate and body mass). This value (23 kJ/d) is 4.5% higher than basal metabolic rate reported by Dohm (1994; also scaled for body mass of 28 g). Maximum aerobic metabolic rate ($\dot{V}O_{2,max}$) is a value predicted for a 28-g mouse with an equation given in figure 4 of Swallow et al. (1998b). At any speed, the metabolic rate is a sum of three components: resting metabolic rate (*black part of bars*), minimum cost of activity (independent of running speed; *hatched*), and incremental cost of running (proportional to speed; *empty*). The bars show proportions of the three components in a mouse running at 0.5 km/h (slow; 7.4 wheel revolutions/min on Fig. 1, *bottom*), 2 km/h (fast; 30 revolutions/min), average speed in mice from control (ctrl; 0.81 km/h or 12.0 revolutions/min) and selected lines (sel; 1.18 km/h or 17.6 revolutions/min), and at estimated maximum aerobic speed (3.36 km/h). In a few of our mice, the maximum speed during a 1-min interval on the wheels was 3.4–3.7 km/h (50–55 revolutions/min), which is close to the predicted maximum aerobic speed. Note that at the average estimated running speeds on wheels (about 1 km/h) about 40% of the cost of locomotion is independent of the running speed. Consequently, running distance over a given period of time (e.g., 24 h) could be increased substantially by increasing speed, with only a small increase in the total cost of activity.

on literature data on metabolic rates of running mice and our observations of voluntary activity on wheels predicts that about 40% of the total cost of locomotor activity in our mice is independent on running speed. This figure is in good agreement with the difference between our estimates of the daily incremental cost of time active (on average 0.70 g/d) and incremental cost of covering distance (on average 0.29 g/d). Note also that, in the last trial without wheels, food consumption was similar to the value predicted by subtracting the cost of time active from the food consumption in the trial with wheels, but much lower than the value predicted by subtracting the cost of running revolutions (Fig. 5). We conclude that total energy budgets

of the mice we studied were more affected by the amount of time active than by the distance traveled. Because mice from selected lines did not spend much more time active, as compared with mice from control lines, the total energy budgets of the former were only marginally higher.

Energy Cost as a Factor Limiting Selection for High Activity

Because the total number of revolutions is a simple product of running speed and time, we might expect that the selection should have led to enhancing both traits. However, the 70% difference in total number of revolutions between selected and control lines was caused primarily by a difference in average running speed, especially in females (Table 1). This result is in concert with the earlier finding that a similar difference between female wild and laboratory mice also resulted from running speed, rather than duration of activity (Dohm et al. 1994).

The fact that selection for high activity has been realized through increased speed, more than time of running, has important consequences for the correlated evolution of behavioral and physiological traits in our system. If high wheel running were to be realized primarily by increased time of activity, then we would expect a substantial increase in total energy expenditure. Consequently, increases in morpho-physiological traits related to energy acquisition, such as gut capacity or maximum energy assimilation, might well evolve. However, if the present trend for increasing primarily velocity continues, then the potential increase of energy expenditure in the selected lines is smaller, and it seems unlikely that constraints related to energy acquisition will be an important factor limiting further evolution of the system.

Related to the foregoing points is our finding that the selected lines have evolved to be smaller in body mass. Because body mass is positively related to energy requirements, the energetic effects of increased activity levels are ameliorated. The net result is that food consumption (or energy assimilation) unadjusted for body mass does not differ significantly between selected and control lines (Table 2).

Conclusions

Mass-adjusted food consumption and assimilation rates are positively related to the amount of wheel running, at the levels of both the variation among individuals and differences between the selected and control lines.

However, the cost of wheel running (number of revolutions) represents a small portion of total energy budgets of the mice, even in the lines selected for high wheel-running activity (7.5%). Therefore, it is unlikely that constraints on energy acquisition will be an important factor limiting further increase of wheel running in the selected lines.

A more general implication of the results is that the energetic consequences of natural selection for higher locomotor activity

depend on how the higher activity actually evolves. A substantial increase in distance traveled may be achieved by increasing running speed, without remarkable increases in energy budget. Increasing the time of activity, however, may be energetically more costly.

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