Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*)

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Swallow, John G., Theodore Garland, Jr., Patrick A. Carter, Wen-Zhi Zhan, and Gary C. Sieck. Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*). *J. Appl. Physiol.* 84(1): 69–76, 1998.—An animal model was developed to study effects on components of exercise physiology of both "nature" (10 generations of genetic selection for high voluntary activity on running wheels) and "nurture" (7–8 wk of access or no access to running wheels, beginning at weaning). At the end of the experiment, mice from both wheel-access groups were significantly lighter in body mass than mice from sedentary groups. Within the wheel-access group, a statistically significant, negative relationship existed between activity and final body mass. In measurements of maximum oxygen consumption during forced treadmill exercise ($\dot{V}O_{2max}$), mice with wheel access were significantly more cooperative than sedentary mice; however, trial quality was not a significant predictor of individual variation in $\dot{V}O_{2max}$. Nested two-way analysis of covariance demonstrated that both genetic selection history and access to wheels had significant positive effects on $\dot{V}O_{2max}$. A 12% difference in $\dot{V}O_{2max}$ existed between wheel-access selected mice, which had the highest mass-corrected $\dot{V}O_{2max}$, and sedentary control mice, which had the lowest. The respiratory exchange ratio at $\dot{V}O_{2max}$ was also significantly lower in the wheel-access group. Our results suggest the existence of a possible genetic correlation between voluntary activity levels (behavior) and aerobic capacity (physiology).

maximum oxygen consumption; wheel running; artificial selection; quantitative genetics; heritability

A WORKING MODEL to describe the complex relationship between habitual physical activity and health-related physical fitness was developed at the International Conference on Exercise, Fitness, and Health (4). According to the model, habitual physical activity can improve health-related fitness traits, which, in turn, may exert positive feedback on habitual activity levels. Individual humans with the highest levels of habitual activity tend to maintain the highest levels of health-related fitness. Similarly, as an individual's level of physical fitness increases, so does the propensity to engage in physical activity. Furthermore, all of the major components of the model are affected by genetics. For example, habitual activity levels (27), as well as health-related fitness traits (3) such as aerobic and anaerobic performance (2), appear to be genetically heritable in humans. However, no information concerning possible genetic correlations (8, 9, 12) between habitual physical activity and physical-fitness traits presently exists. An understanding of the underlying genetics of habitual activity will help answer questions concerning the relationship between activity and health. For instance, do individuals with a genetic propensity for high levels of physical activity also tend to have high levels of aerobic fitness?

We propose the use of an animal model to study simultaneously the effects of both "nature" (genetic endowment for high activity) and "nurture" (access to a running wheel) on physical-fitness traits. Animal models have been widely used to study physiological effects of exercise training. For example, rodents given access to running wheels will display significant amounts of spontaneous activity. Individual variation in wheel-running behavior is substantial; some animals will run many kilometers per day, whereas others engage in almost no activity (e.g., Refs. 11 and 14). Furthermore, wheel-running activity has been shown to elicit a variety of physiological adaptations to training [e.g., increased maximum O_2 consumption ($\dot{V}O_{2max}$) and running economy (22); muscle enzyme activity (28); and vascular adaptations (29)]. In addition, animal models using wheel running have provided insight into the effects of exercise on health and longevity (e.g., Ref. 21).

Artificial selection is one of several tools that can be applied with animal models to separate genetic and environmental influences on particular phenotypic traits (12, 17). The goal of selective breeding is often to change the mean phenotype of a defined population compared with a control population. Selective breeding should result in changes in allele frequencies for all genes associated with the phenotype under selection; allele frequencies of genes unrelated to the selected phenotype are expected to remain unchanged, except for possible effects of linkage or random genetic drift. In the present study, we used selective breeding to create four replicate lines of mice with high activity levels, compared with four random-bred control lines (30); 10 generations of selective breeding resulted in an ~75% increase in the activity level of males (the sex used in this study) compared with males from control lines.

Selection for a single trait may also result in correlated changes in other traits (12, 15, 17). Because high levels of habitual activity may require high endurance and aerobic capacity, we hypothesized that selection for high levels of activity would be accompanied by a concomitant increase in aerobic capacity (see also Refs. 10 and 18). $\dot{V}O_{2max}$ during exercise appears to be genetically heritable (2) and thus capable of responding to selection in either a direct or correlated manner. In a two-way design, we studied the effects of both genetic selection history and exercise history (access to run-

ning wheels) on body mass, $\dot{V}_{O_2max}$, and respiratory exchange ratio (RER).

MATERIALS AND METHODS

Animal husbandry and breeding design. The mice used in this study were sampled from an artificial selection experiment for increased voluntary activity levels on running wheels, including four replicate selected lines and four randomly bred control lines (30). These mice are the result of 10 generations of within-family selection for total wheel-running activity. Each generation of mice was housed individually with access to running wheels and scored for activity over a 6-day period; selection was based on the total number of revolutions run on *days 5* and *6*. After 10 generations of selective breeding, males from the selected lines were ~75% more active than males from the control lines. The original progenitors of these lines were outbred HSD:ICR mice (see Refs. 8–11 for strain history) purchased from Harlan Sprague Dawley (Indianapolis, IN) in 1993.

The mice studied herein were from second litters; their siblings (first litters) were part of the routine selection protocol and were measured for wheel-running activity at 6–8 wk of age. The parents had first been mated at 8 wk and were then remated at ~13 wk of age by placing each male with its mate from the first pairing the day after the first litter was weaned. Males were removed 15–18 days after pairing; births began 19 days after pairing. From 19 to 33 days after pairing, pregnant females were checked daily between 1600 and 1800. At 21 days of age, offspring were weaned from the dam, weighed, and toe clipped for individual identification.

One male was selected at random from each family for measurement to ensure statistical independence of data points. Only males were studied to eliminate possible sex-related variation both in behavioral (females tend to run more on the wheels) and physiological traits (6, 8–10). Twelve mice were assigned to each of four measurement groups: sedentary control, wheel-access control, sedentary selected, and wheel-access selected. The sedentary mice were housed in groups of four in standard clear plastic cages (27 × 17 × 12.5 cm) with metal tops and wood shavings in a temperature-controlled room (~22°C) with a constant 12:12-h light-dark cycle centered at 1400 (CST). Water and food [Harlan Teklad Laboratory Rodent Diet (W)-8604] were available ad libitum. The wheel-access mice were housed with four siblings until the following day, and then they were housed individually with access to running wheels. As a result of variation in birth date, time of access to wheels (before measurement of mass and $\dot{V}_{O_2max}$) ranged between 51 and 61 days.

Voluntary wheel-running behavior. In the wheel-access group, voluntary activity was monitored every day for each mouse from 22 days of age until the day before measurement of peak exercise metabolism (73–83 days of age). Voluntary wheel running was measured on Wahman-type activity wheels [as described (11): 1.12-m-circumference, 10-cm-wide running surface of 10-mm mesh bounded by clear Plexiglas walls; Lafayette Instruments, Lafayette, IN; model 86041 with modifications]. Normal housing cages were attached to the wheels by a 7.7-cm-diameter hole in the cage side so that mice had continuous access to activity wheels. Attached to each wheel was a photocell counter, which was interfaced to an IBM-compatible personal computer. Customized software from San Diego Instruments (San Diego, CA) measured the number of clockwise and counterclockwise revolutions during every 1-min interval for each wheel. Data were downloaded every 24 h.

Peak exercise metabolic rate. $\dot{V}_{O_2max}$ and maximum carbon dioxide production ($\dot{V}_{CO_2max}$) were measured on 2 consecutive days via an incremental step protocol on a motorized treadmill (8, 10, 11, 14, 19). Measurements were made first in the sedentary group and then in the wheel-access group. All measurements were done from July 24 to 27 between 0900 and 1900. The wheel-access group continued to be housed with access to wheels during this period, but running activity was not recorded.

The testing protocol was as follows. Two minutes of baseline data were collected on ambient air. A mouse was then placed in a small Plexiglas chamber held just above the surface of the treadmill belt, thus allowing inflow of room air. Chamber inner dimensions were 13 × 6.3 × 5 cm at the highest and 13 × 6.3 × 2 cm at the lowest portion of the wedge-shaped extension over an electrified grid. Mice were first placed in the chamber while the treadmill was stopped, and resting O_2 consumption ($\dot{V}_{O_2}$) was recorded for 1.5–2 min. The treadmill was then started at an initial speed of 1.5 km/h. Mice were induced to run by being prodded with a straightened paper clip inserted through a hole at the rear of the chamber and/or by a mild electric current (50–110 V, 3–12 mA) provided through a horizontal grid of twelve 2-mm bars spaced 5 mm apart at the end of the moving belt. Treadmill speed was then increased every 2 min by 0.5 km/h. All mice reached at least 2.5 km/h; the maximum speed attained by any mouse in this study was 4.0 km/h. Trials were ended when $\dot{V}_{O_2}$ failed to increase with increasing speed and/or the mouse failed to keep pace with the treadmill. $\dot{V}_{O_2}$ generally decreased before a trial was ended (i.e., while the mouse was still running). After the treadmill was stopped, mice were left in the chamber for 1.5–2 min. Mice were then removed from the chamber, and baseline data were again recorded for 2 min. Body mass of each animal was recorded on the first day of measurement. Time of day, speed at which the trial ended, and a subjective assessment of run quality (5 categories from poor to excellent) were recorded at the end of each trial. All measurements were blind with respect to selection history and were taken by one individual (J. G. Swallow).

Gas exchange was monitored with an open-circuit respirometry system. Air was drawn from the running chamber via a series of eight ports (each 3 mm diameter) in its top, through a column of Drierite for removal of water vapor, and then passed through a thermal mass-flow controller (series 840 Side=Trak, Sierra Instruments) set at 2,500 ml/min STPD. This flow rate ensured rapid chamber washout; time to initial response was <5 s. Excurrent air was analyzed continuously by an Ametek (Applied Electrochemistry) S-3A/II O_2 analyzer and an Ametek (Applied Electrochemistry) CD-3A CO_2 analyzer. Effective volume of the system was determined separately for O_2 (604 ml) and CO_2 (630 ml), and analysis software made "instantaneous" corrections for chamber washout (1) because standard equations assume steady-state (equilibrium) conditions. Instantaneous corrections are minor with a rapid washout rate, as in our system (see RESULTS; Refs. 8, 10, and 11).

Our data-acquisition program recorded values for O_2 and CO_2 each second as the average of 20 consecutive readings and wrote the data to disk. The program also allowed us to put marks in the file (used to indicate baseline, mouse in chamber without treadmill running, and speed of treadmill). A data-analysis program corrected for baseline drift by using linear regression. CO_2 production ($\dot{V}_{CO_2}$) values were calculated by using *Eq. 2* in Ref. 16

$$\dot{V}_{CO_2} = (\dot{V}_E)(F_{E_{CO_2}} - F_{I_{CO_2}})$$

and then $\dot{V}O_2$ values were calculated by using *Eq. 3b* in Ref. 33

$$\dot{V}O_2 = [(\dot{V}E)(FI_{O_2} - FE_{O_2}) - (\dot{V}CO_2 \times FI_{O_2})]/(1 - FI_{O_2})$$

where $\dot{V}CO_2$ is expressed as milliliters CO_2 per hour (STPD); $\dot{V}O_2$ is expressed as milliliters O_2 per hour (STPD); FI is fractional concentration of gas in inspired air; FE is fractional concentration of gas in expired air; and $\dot{V}E$ is flow rate (ml/h; STPD).

The highest 1-min period of $\dot{V}O_2$ and $\dot{V}CO_2$ was determined separately (both steady-state and instantaneous) for each treadmill run. The higher of the two measurements was taken as $\dot{V}O_{2max}$ and $\dot{V}CO_{2max}$, respectively. RER and speed at which $\dot{V}O_{2max}$ and $\dot{V}CO_{2max}$ were first attained (determined from marks in the data file) were also recorded.

Statistical analyses. Wheel running (over first 2 wk of exposure to wheels and over the last 6 wk of exposure to wheels), body mass at weaning, body mass within the wheel-access group, and $\dot{V}O_{2max}$ within the wheel-access group were analyzed by nested one-way analysis of variance (ANOVA) with type III sums of squares in the Statistical Analysis System General Linear Models (SAS GLM) procedure. Line type (selected vs. control) was used as the grouping variable; replicated line (n = 8 total) was nested within line type. Number of toes clipped for identification and an index of wheel rotational resistance were used as covariates in analyses of wheel running. Average number of revolutions run per day (rev/day) during the last week of exposure to wheels was used as a covariate in the analysis of final body mass within the wheel-access group. Average number of rev/day during the last week of exposure to wheels as well as body mass and trial quality were used in the analysis of $\dot{V}O_{2max}$ within the wheel-access group.

Body mass, $\dot{V}O_{2max}$, $\dot{V}CO_{2max}$, RER, running performance (speed at $\dot{V}O_{2max}$, speed at $\dot{V}CO_{2max}$, maximum speed attained in trial), and trial quality were analyzed by a nested two-way ANOVA with type III sums of squares in the SAS GLM procedure. Exercise group (sedentary vs. wheel-access) and line type (selected vs. control) were the grouping factors; replicate line was nested within line type. Body mass was included as a covariate in all analyses of exercise metabolism. Models were tried both with and without trial quality as a covariate in analyses of $\dot{V}O_{2max}$ and $\dot{V}CO_{2max}$; results from both analyses (with and without trial quality) are presented. Trial quality, but not body mass, was used in the analyses of treadmill-running performance. The foregoing are mixed models with both random (exercise group) and fixed (line type and line) factors. Therefore, effects of line type were tested over the mean squares of line-within-line type, and effects of exercise group were tested over the mean squares of the interaction of exercise group × line-within-line type. Adjusted means were calculated by using the LSMEANS command in the SAS GLM procedure; all covariates in the model, regardless of statistical significance, were used to calculate adjusted means.

RESULTS

Wheel running. Figure 1 shows the mean rev/day by mice from the selected and the control lines as a function of age. During the first 2 wk of exposure to the running wheels, when mice were 22–36 days of age, mice from selected lines ran significantly more rev/day than did mice from the control lines [F = 6.83; degrees of freedom (df) = 1,6; P = 0.0399]. Mice from both selected and control lines exhibited a temporal trend for increased wheel running over the first 6 wk of exposure to wheels, after which activity levels declined. Although mice from selected lines continued to have higher mean values of rev/day from *weeks 3* to *8*, this difference was no longer statistically significant. Number of toes clipped and wheel resistance were never significant in any of the analyses.

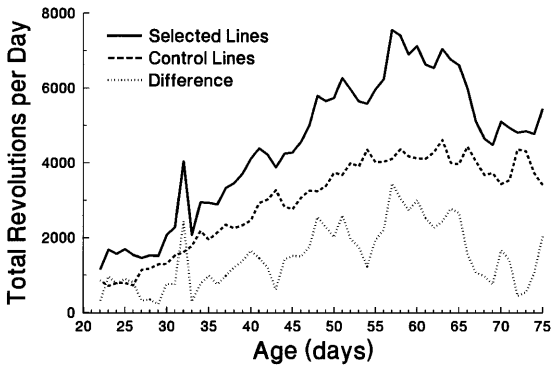

Fig. 1. Average daily running distance for mice from selected (n = 23) and control (n = 24) lines. One revolution equals 1.12 m.

As recorded in this study, total wheel running can be broken into two components: number of 1-min intervals during which any activity occurred and mean revolutions per minute (rpm) during those minutes of activity (Table 1). Mice from selected and control lines did not differ significantly in the number of 1-min intervals run during the first 2 wk of wheel exposure (F = 1.77; df = 1,6; P = 0.2315). Instead, mice from the selected lines ran at a higher rpm compared with controls (F = 6.21; P = 0.0471). In both groups, the temporal increase in total revolutions resulted from increases in both rpm and number of 1-min intervals (Table 1). As with total rev/day, the difference between the groups was no longer statistically significant after the first 2 wk of exposure for either 1-min intervals or rpm.

Body mass data are presented in Table 2. Body mass of the four groups did not differ significantly at 21 days of age (F = 0.05; df = 1,6; P = 0.8294). By the end of the wheel trial, however, a two-way analysis of covariance (ANCOVA) indicated that mice from the wheel-access group averaged >4 g lighter than mice from the

Table 1. *Wheel-running data*

Variable	Group: Control (n = 12)	Group: Selected (n = 11)	Selected/Control
Total revolutions/day			
First 2 wk of exposure*	1,326 ± 167.1	1,985 ± 176.6	1.50
Last week of exposure	3,940 ± 773.7	5,228 ± 817.5	1.33
1-min Intervals/day			
First 2 wk of exposure	162.6 ± 13.89	190.5 ± 14.67	1.17
Last week of exposure	278.0 ± 31.27	290.3 ± 30.03	1.04
Average rpm			
First 2 wk of exposure*	7.6 ± 0.59	9.8 ± 0.62	1.29
Last week of exposure	13.3 ± 1.52	16.3 ± 1.68	1.23

Values are adjusted means ± SE; n, no. of animals. rpm, Revolutions/min. *$P < 0.05$ for effect of line type (i.e., genetic selection) in a nested 1-way analysis with number of toes clipped and wheel resistance as covariates.

Table 2. *Body mass and age of mice from 4 measurement groups*

Variable	Group			
	Sedentary control ($n = 11$)	Wheel-access control ($n = 12$)	Sedentary selected ($n = 12$)	Wheel-access selected ($n = 11$)
Body mass at weaning (21 days), g				
Mean	11.3 ± 1.23	11.2 ± 1.75	10.9 ± 2.75	10.8 ± 2.01
Range	9.8–13.6	8.7–14.0	7.3–15.6	7.2–13.4
Body mass when taken off wheels, g*				
Mean	37.7 ± 2.76	34.2 ± 2.54	37.4 ± 3.19	32.5 ± 4.17
Range	34.1–41.6	30.2–39.1	32.1–43.3	27.1–39.4
Age when taken off wheels, days				
Mean	82.7 ± 1.19	79.6 ± 2.39	82.1 ± 1.16	79.3 ± 1.19
Range	80–84	75–83	81–85	77–81

Values are means ± SE; n, no. of animals. *$P < 0.05$ for effect of exercise group in a nested 2-way analysis without covariates.

sedentary group ($F = 11.23$; df = 1,6; $P = 0.0154$); however, body mass of mice from selected lines did not differ significantly from controls ($F = 3.34$; df = 1,6; $P = 0.1176$).

For the exercise group, analysis of body mass was repeated with average rev/day during the last week of wheel exposure as a covariate. In this analysis, amount of wheel running had a significant negative effect on body mass ($F = 8.34$; df = 1,14; $P = 0.0119$; Fig. 2). After adjustment for total running activity, differences between selected and control lines were still nonsignificant ($F = 0.76$; df = 1,6; $P = 0.4178$). The analysis was also repeated with rev/day averaged over longer periods of time (i.e., averaged over the last 2–6 wk) as a covariate. All were significant predictors of body mass, but average rev/day during the last week was the best predictor, as indicated by the highest model r^2.

Metabolic responses. Between trial days, individual mice displayed repeatable variation in trial quality (cooperativity) during the treadmill test ($r = 0.489$; $P = 0.001$), and trial quality was significantly higher on the second trial (paired $T = 2.14$; df = 1,43; 2-tailed, $P = 0.038$). Because trial quality can influence whether an animal reaches $\dot{V}O_{2max}$, it has often been used to assess whether a particular run should be included in analyses (e.g., Ref. 5). To our knowledge, however, trial quality has not been used as a covariate in analyses, even though it may influence metabolism. Therefore, we tried analyses with and without trial quality as a covariate, both to allow comparability with previous studies and to correct for possible effects of run quality.

Consistent with previous studies in our laboratory (8, 10, 11, 14), steady-state and instantaneous values of $\dot{V}O_{2max}$ were highly correlated ($r = 0.980$), with the latter averaging 4% higher. Here, only instantaneous values were presented; regardless of which values are analyzed, the basic conclusions remain unchanged.

In a two-way ANCOVA, mice from the selected lines had significantly higher $\dot{V}O_{2max}$ than did mice from control lines ($F = 10.13$; df = 1,6; $P = 0.0190$); wheel-access mice also had higher values of $\dot{V}O_{2max}$ than did sedentary mice, although this difference was only marginally significant ($F = 5.88$; df = 1,6; $P = 0.0515$). The interaction term between line type and exercise group was not statistically significant ($F = 1.72$; df = 1,6; $P = 0.2378$). Figure 3 shows instantaneous $\dot{V}O_{2max}$ as a function of body mass; as expected, body mass was a significant predictor of $\dot{V}O_{2max}$ ($F = 26.51$; df = 1,26; $P = 0.0001$). Trial quality was not a significant predictor of $\dot{V}O_{2max}$ ($F = 1.68$; df = 1,26; $P = 0.2058$).

In the reduced model of $\dot{V}O_{2max}$ without trial quality, significance levels for both line type ($F = 10.36$; df = 1,6; $P = 0.0185$) and exercise group ($F = 7.63$; df = 1,6; $P = 0.0328$) were similar (Table 3). Again, the interaction term was not statistically significant ($F = 1.26$; df = 1,6; $P = 0.3048$), and body mass was a significant predictor ($F = 24.34$; df = 1,26; $P = 0.0001$).

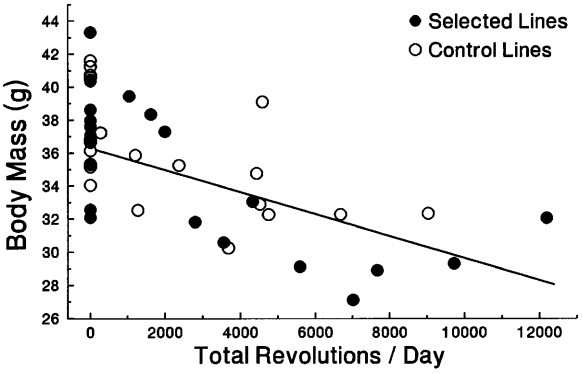

Fig. 2. Body mass of selected and control mice measured at end of wheel access (75–85 days of age) in relation to daily running distance (average over last week of exposure to wheels). Mice were weaned at 21 days of age and given access to wheels at 22 days of age. Mice without access to wheels ("sedentary") are plotted with zero revolutions/day. Regression line is for wheel-access mice only ($r^2 = 0.35$).

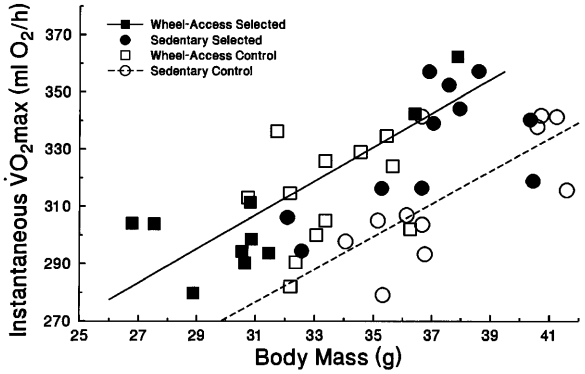

Fig. 3. Relationship between maximum oxygen consumption ($\dot{V}O_{2max}$) during forced treadmill exercise and body mass for 4 groups of mice. Regression lines are for wheel-access selected (solid line; $r^2 = 0.67$) and sedentary control (dashed line; $r^2 = 0.50$) animals; mass-corrected $\dot{V}O_{2max}$ of former averages is 12% higher than for sedentary controls (Table 3: adjusted means of whole-animal values).

Table 3. *Instantaneous gas-exchange data*

Variable	Group			
	Sedentary control ($n = 11$)	Wheel-access control ($n = 12$)	Sedentary selected ($n = 11$)	Wheel-access selected ($n = 10$)
Body mass at $\dot{V}O_{2max}$, g[b,d]				
Mean	37.7 ± 0.83	33.4 ± 0.50	36.9 ± 0.82	31.2 ± 1.11
Range	34.1–41.6	30.8–36.3	32.1–40.5	26.8–37.9
Age at $\dot{V}O_{2max}$, days[d]				
Mean	83.2 ± 0.36	81.9 ± 0.69	82.6 ± 0.37	81.6 ± 0.40
Range	80–85	77–85	81–86	79–83
$\dot{V}O_{2max}$, ml/h[a,b,e]				
Mean	314.8 ± 6.72	313.0 ± 5.05	331.0 ± 6.53	308.0 ± 8.01
Range	279.0–341.6	284.0–336.1	294.3–357.1	279.7–362.2
Adjusted mean	296.3 ± 5.64	322.2 ± 4.58	317.8 ± 5.16	333.2 ± 6.58
$\dot{V}O_{2max}$, ml·kg^{-1}·min^{-1} [a,c,d]				
Mean	139.3 ± 2.33	156.4 ± 3.02	150.0 ± 2.61	165.4 ± 3.72
Range	126.4–155.2	138.8–176.6	131.3–161.2	155.6–189.3
$\dot{V}CO_2$ at $\dot{V}O_{2max}$, ml/h[e]				
Mean	332.2 ± 11.00	310.5 ± 6.28	342.6 ± 8.88	297.8 ± 9.95
Range	275.9–391.8	262.5–338.6	268.7–366.6	238.0–356.0
Adjusted mean	313.0 ± 6.38	320.8 ± 5.18	328.8 ± 5.83	326.7 ± 7.44
RER ($\dot{V}CO_2/\dot{V}O_2$) at $\dot{V}O_{2max}$[c,d]				
Mean	1.05 ± 0.024	0.99 ± 0.018	1.04 ± 0.018	0.97 ± 0.024
Range	0.91–1.21	0.84–1.08	0.88–1.09	0.85–1.07
$\dot{V}CO_{2max}$, ml/h[e]				
Mean	341.5 ± 9.68	326.0 ± 7.39	351.7 ± 9.04	312.0 ± 12.25
Range	289.3–398.7	272.6–378.9	295.1–390.6	248.2–394.5
Adjusted mean	326.0 ± 8.74	344.8 ± 7.09	346.2 ± 7.99	341.0 ± 10.20
Speed at $\dot{V}O_{2max}$, km/h[f]				
Mean	1.9 ± 0.17	2.3 ± 0.27	1.9 ± 0.15	2.1 ± 0.20
Range	1.5–3.0	1.5–4.0	1.5–3.0	1.5–3.5
Adjusted mean	1.8 ± 0.21	2.2 ± 0.17	1.9 ± 0.20	2.1 ± 0.25
Maximum speed attained, km/h[f]				
Mean	3.0 ± 0.00	3.2 ± 0.16	2.8 ± 0.08	3.3 ± 0.13
Range	3.0–3.0	2.5–4.0	2.5–3.0	3.0–4.0
Adjusted mean	3.1 ± 0.10	3.1 ± 0.09	3.0 ± 0.10	3.1 ± 0.12
Trial quality[b,d]				
Mean	3.1 ± 0.09	3.8 ± 0.33	3.1 ± 0.25	4.1 ± 0.28
Range	3.0–4.0	2.0–5.0	2.0–4.0	3.0–5.0

Values are means and adjusted means reported ± SE; n, no. of animals. $\dot{V}O_2$, O_2 consumption; $\dot{V}O_{2max}$, maximal $\dot{V}O_2$; $\dot{V}CO_2$, CO_2 production; $\dot{V}CO_{2max}$, maximal CO_2 production; RER, respiratory exchange ratio. [a]$P < 0.05$ for effects of line type (i.e., genetic selection); [b]$P < 0.05$ for effects of exercise group; [c]$P < 0.01$ for effects of exercise group; [d]adjusted means not calculated because no covariates were used in analysis; [e]adjusted means calculated with body mass as a covariate; [f]adjusted means calculated with trial quality but not body mass as a covariate.

As with body mass, the analysis of $\dot{V}O_{2max}$ was repeated within the wheel-access group with average rev/day as an additional covariate during the last week of wheel exposure. Differences between mice from the selected and control lines were no longer statistically significant ($F = 1.34$; df = 1,6; $P = 0.2918$). Amount of wheel running did not predict individual variation in $\dot{V}O_{2max}$ ($F = 1.07$; df = 1,11; $P = 0.3242$), but body mass did ($F = 16.57$; df = 1,26; $P = 0.0018$).

The treadmill speed at which $\dot{V}O_{2max}$ was attained averaged slightly higher than 2 km/h (Table 3) and did not differ significantly between either selected and control lines ($F = 0.44$; df = 1,6; $P = 0.5324$) or wheel-access and sedentary mice ($F = 0.01$; df = 1,6; $P = 0.9264$). Similarly, maximum speed attained on the treadmill averaged slightly higher than 3 km/h (Table 3) and did not differ between either selected and control lines ($F = 0.62$; df = 1,6; $P = 0.4619$) or wheel-access and sedentary mice ($F = 0.16$; df = 1,6; $P = 0.7013$). In the foregoing analyses, the subjective measurement of trial quality was a significant, positive predictor of the speed at which $\dot{V}O_{2max}$ was attained ($F = 7.61$; df = 1,27; $P = 0.0103$) as well as the maximum speed attained on the treadmill ($F = 27.81$; df = 1,27; $P = 0.0001$). We repeated the analyses without trial quality as a covariate. Speed at which $\dot{V}O_{2max}$ was attained still did not differ significantly in relation to selection ($F = 0.08$; df = 1,6; $P = 0.7874$) or exercise group ($F = 1.58$; df = 1,6; $P = 0.2549$). Maximum speed attained in a trial did not differ in relation to selection ($F = 0.01$; df = 1,6; $P = 0.9247$) but was higher in the wheel-access mice ($F = 8.34$; df = 1,6; $P = 0.0278$).

No significant difference in $\dot{V}CO_{2max}$ was observed between mice from either selected and control lines ($F = 1.02$; df = 1,6; $P = 0.3514$) or wheel-access and sedentary groups ($F = 0.33$; df = 1,6; $P = 0.5858$). Again, the interaction term between line type and exercise group was not statistically significant ($F = 2.77$; df = 1,6; $P = 0.14$). Body mass, but not trial quality, was a significant predictor of $\dot{V}CO_{2max}$. Not surprisingly, $\dot{V}CO_{2max}$ was significantly higher than $\dot{V}CO_2$ at $\dot{V}O_{2max}$ (Table 3; paired $T = 7.50$; df = 1,43; $P = 0.0001$). No group differences ($F < 0.05$; df = 1,6; $P > 0.5$) existed for the speed at which $\dot{V}CO_{2max}$ was at-

tained, whether or not trial quality was used as a covariate. In the reduced model of $\dot{V}CO_{2max}$ without trial quality, effects of neither line type (F = 1.02; df = 1,6; P = 0.3519) nor exercise group (F = 0.34; df = 1,6; P = 0.5807) were statistically significant (Table 3). The interaction term also was not statistically significant (F = 2.77; df = 1,6; P = 0.1470).

In the two-way ANCOVA, access to running wheels significantly reduced the RER at $\dot{V}O_{2max}$ (F = 7.15; df = 1,6; P = 0.0368); however, genetic selection history did not affect RER at $\dot{V}O_{2max}$ (F = 0.42; df = 1,6; P = 0.5417), and neither body mass nor trial quality was a significant predictor of RER. In a reduced model with neither body mass nor trial quality as a covariate, results were qualitatively similar; access to running wheels significantly reduced RER (F = 22.42; df = 1,6; P = 0.0032; Table 3), but genetic selection had no effect (F = 0.66; df = 1,6; P = 0.4482). As might be expected, RER at $\dot{V}CO_{2max}$ (grand mean = 1.11) was significantly higher than RER at $\dot{V}O_{2max}$ (grand mean = 1.02; paired T = 10.28; df = 1,43; P = 0.0001).

Mice with access to activity wheels had significantly higher subjective measurements of trial quality than did sedentary mice (2-way ANOVA: F = 8.19; df = 1,6; P = 0.0287; Table 3). Thus wheel-access mice were more cooperative on the treadmill. Trial quality did not differ significantly in relation to selection history (F = 0.77; df = 1,6; P = 0.4142).

DISCUSSION

This experiment clearly demonstrates an effect of nature on $\dot{V}O_{2max}$. The average difference in $\dot{V}O_{2max}$ between mice from genetically selected and control lines is 6% (Table 3; adjusted means of whole-animal values). Thus 10 generations of selection for a single behavioral trait, voluntary activity on running wheels, resulted not only in a shift in mean activity levels but also in a correlated response in $\dot{V}O_{2max}$. Our results suggest a mechanistic link between habitual activity levels and aerobic capacity: some of the genes that influence wheel-running behavior must also influence $\dot{V}O_{2max}$.

Nurture had an effect on $\dot{V}O_{2max}$ that was similar in magnitude to the effect of nature (Table 3; 6% difference in adjusted means of whole-animal values). Several previous studies of rodents have also shown that voluntary wheel-running activity results in increased aerobic capacity. MacNeil and Hoffman-Goetz (23) reported that, after 8 wk of voluntary wheel running, male house mice (C3H/He) had 21% higher $\dot{V}O_{2max}$ values compared with sedentary controls. Overton et al. (25) found a 20% difference between sedentary and wheel-access female rats (SP-SHR) but only a 9% difference in similarly treated male rats after 4 wk of voluntary exercise. Lambert and Noakes (22) found a 9% difference in $\dot{V}O_{2max}$ between wheel-access and sedentary male rats (Long-Evans) after a 12-wk exposure.

Several protocol differences may explain the smaller training effect that we observed (6% increase in $\dot{V}O_{2max}$) compared with other studies. First, the animals in this study were neither prescreened for running ability (e.g., Ref. 25) nor divided into activity categories before analysis (e.g., Ref. 22); therefore, the selected group mean (Tables 1–3) includes data from mice that exhibited low levels of activity. Second, animals in our study were given access to wheels at a younger age (22 days) and smaller body mass (10 g), which may have limited early activity (see Fig. 1).

Finally, the way $\dot{V}O_2$ data are reported may affect apparent differences among groups. Among species of mammals, exercise $\dot{V}O_{2max}$ has been empirically determined to scale approximately as mass$^{0.8}$. Alternatively, $\dot{V}O_{2max}$ expressed per unit body mass scales as mass$^{-0.2}$ (20, 31). Although this relationship has not been extensively studied within species of mammals, $\dot{V}O_{2max}$ expressed per unit body mass clearly shows a significant negative relationship with body mass within laboratory house mice (Fig. 4); the smallest individuals have the highest mass-specific rates of $\dot{V}O_2$. Therefore, expressing the ratio of $\dot{V}O_2$ to unit body mass inflates the group difference in comparisons between lighter, trained and heavier, untrained animals. In the present study, when $\dot{V}O_{2max}$ is expressed per unit body mass (Table 3), the apparent magnitude of the difference between wheel-access and sedentary mice doubles to 12% because wheel-access mice are >4 g lighter than sedentary mice.

In our analysis, the interaction between line type and exercise group was not statistically significant (P > 0.2). Lack of interaction implies that the effects of selection and exercise group are independent and additive, not dependent on genotype. However, ANOVA has relatively low power to detect genotype-by-environment interaction (32). To explore a possible genotype-dependent training response in more detail, we analyzed the sedentary group and the wheel-access group separately with one-way ANCOVA (body mass and trial quality as covariates). Analysis of the sedentary group indicated that $\dot{V}O_{2max}$ was significantly higher in mice from selected lines compared with mice from control lines (F = 11.34; df = 1,6; P = 0.0151). Analysis of the

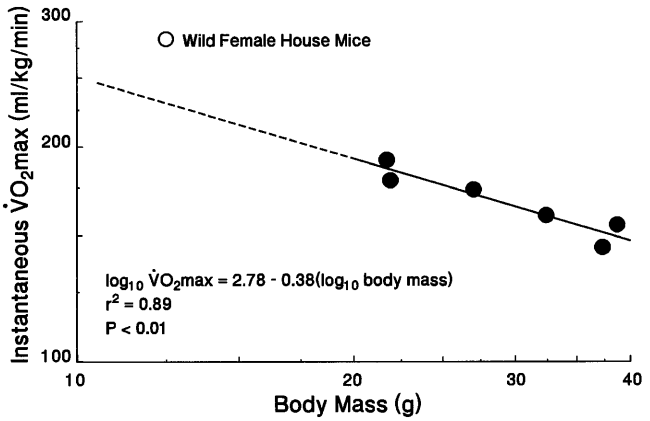

Fig. 4. Mass-specific $\dot{V}O_{2max}$ of house mice (measured during forced treadmill exercises) in relation to body mass. Regression line is for laboratory house mice only (solid circles); dashed line is an extrapolation beyond data. Sources of mean values were as follows: males and females (8); wild and laboratory females (11); males (Ref. 14, multiplied by 1.04 to correct for chamber washout); present study for all wheel-access and all sedentary mice.

wheel-access group, however, indicated that $\dot{V}O_{2max}$ did not differ significantly between selected and control lines ($F = 2.21$; df = 1,6; $P = 0.1881$). Thus trainability may differ between line types. If this difference is real, then one possible explanation is that the control mice were running at a higher relative intensity (i.e., at a higher percentage of $\dot{V}O_{2max}$), which, over the course of the experiment, led to a larger relative increase in $\dot{V}O_{2max}$. In any case, future studies with larger sample size will be required to test the hypothesis that sensitivity to training is genotype dependent in these mice.

Our estimate of $\dot{V}O_{2max}$ is similar to others previously reported (Fig. 4). Our empirical results also closely match those predicted by an equation developed by Fernando et al. (13) to predict $\dot{V}O_2$ of mice running on a treadmill, on the basis of body mass and running velocity

$$\dot{V}O_{2\,max}\ (\text{ml/min}) = 0.127 \times \text{mass (g)} + 0.040 \times \text{running speed (m/min)} - 0.974$$

For a 36-g mouse (average mass of mice in our study) running at 2 km/h (average speed at $\dot{V}O_{2max}$), this equation predicts 295 vs. 317 ml/h found in our study.

In this experiment, animals that had access to running wheels had a significantly lower RER at $\dot{V}O_{2max}$ compared with sedentary animals (Table 3). Selection history did not affect RER, even though animals from selected lines had higher aerobic capacity. It is tempting to ascribe the lowered RER in the wheel-access animals to a training adaptation involving increased fat metabolism. At this workload, however, hyperventilation, causing increased $\dot{V}CO_2$, confounds interpretation of RER with respect to fat oxidation, particularly because run quality differed significantly between sedentary and wheel-access mice. To our knowledge, no other estimate of RER at $\dot{V}O_{2max}$ is available for house mice. Our estimates of RER (Table 3) are somewhat low but within the range of values reported for rats (e.g., Refs. 5, 7, 24).

As noted by others working with rats (22), we found that mice with access to activity wheels were significantly more cooperative during treadmill trials than were sedentary mice. The subjective measurement of trial quality was not significantly correlated with $\dot{V}O_{2max}$ (see also Ref. 6 on serum corticosterone levels during treadmill exercise), but it was positively correlated with both speed at which $\dot{V}O_{2max}$ was attained and the maximum speed attained before termination of the $\dot{V}O_{2max}$ trial. Mice that received higher subjective scores for trial quality were those that were able to run without much external stimulation (i.e., without much electrical stimulation or manual prodding). Fernando et al. (13) noted that volitional running was easily maintained in mice without the use of electrical stimulation at speeds below 1.2 km/h (see also Ref. 6). In our study, the starting speed, 1.5 km/h, was above the speed for volitional running; thus all mice in our study required some external stimulation.

As reported by others (e.g., Refs. 11, 14, 22, 28, 29), the present study revealed large individual variation in average daily running distance (Fig. 2). As expected, mice from lines that had been subjected to 10 generations of selection for high activity levels (30) ran significantly more total rev/day than did mice from unselected control lines. The significant difference in total activity during the first 2 wk between selected and control lines is caused mainly by the mice from the selected lines running faster, not by their running during a greater fraction of the day. Over the course of the experiment, total rev/day increased as a result of increases in both the number of 1-min intervals of activity and average rpm (Fig. 1; Table 1).

Previous studies of both house mice (23) and rats (22, 28) show that male animals with access to wheels gain less weight than do sedentary controls, even with similar or higher rates of food intake (however, see Ref. 26). We observed a similar pattern in body mass changes. Body masses of the different groups were not different at the beginning of the experiment, but after 7–8 wk of access to the wheels the wheel-access mice were significantly lighter than sedentary mice (Table 1). Furthermore, within the wheel-access group, total daily wheel running averaged over the last week of exposure to wheels was negatively correlated with final body mass (Fig. 2). The mechanism underlying the difference in mass gain is unknown.

In summary, we developed and tested an animal model to study the effects on aerobic exercise metabolism of both nature and nurture. Consistent with previous studies (22, 25, 28, 29), we found that access to wheels significantly reduced body mass, reduced RER at $\dot{V}O_{2max}$, and increased $\dot{V}O_{2max}$. Our results are unique in that we demonstrated a significant effect of genetic selection history on $\dot{V}O_{2max}$. These results suggest that habitual activity levels and $\dot{V}O_{2max}$ are positively genetically correlated in house mice. Evidence for genotype-sensitive training effects, however, was equivocal.

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