Voluntary Exercise and Its Effects on Body Composition Depend on Genetic Selection History

Derrick L. Nehrenberg[1], Kunjie Hua[1], Daria Estrada-Smith[1], Theodore Garland Jr[2] and Daniel Pomp[1,3,4]

Little is known about how genetic variation affects the capacity for exercise to change body composition. We examined the extent to which voluntary exercise alters body composition in several lines of selectively bred mice compared to controls. Lines studied included high runner (HR) (selected for high wheel running), M16 (selected for rapid weight gain), Institute of Cancer Research (ICR) (randomly bred as control for M16), M16i (an inbred line derived from M16), HE (selected for high percentage of body fat while holding body weight constant), LF (selected for low percentage of body fat), C57BL/6J (common inbred line), and the F1 between HR and C57BL/6J. Body weight and body fat were recorded before and after 6 days of free access to running wheels in males and females that were individually caged. Total food intake was measured during this 6-day period. All pre- and postexercise measures showed significant strain effects. While HR mice predictably exercised at higher levels, all other selection lines had decreased levels of wheel running relative to ICR. The HR × B6 F1 ran at similar levels to HR demonstrating complete dominance for voluntary exercise. Also, all strains lost body fat after exercise, but the relationships between exercise and changes in percent body were not uniform across genotypes. These results indicate that there is significant genetic variation for voluntary exercise and its effects on body composition. It is important to carefully consider genetic background and/or selection history when using mice to model effects of exercise on body composition, and perhaps, other complex traits as well.

Obesity (2009) **17,** 1402–1409. doi:10.1038/oby.2009.51

INTRODUCTION

Obesity is determined by the balance between energy intake and energy expenditure, as regulated via a multitude of metabolic processes (1). Because energy expenditure from physical activity has the potential to alter this balance, considerable effort has been directed at identifying how physical activity prevents weight gain and also inhibits weight gain after weight loss (2). One basic problem in identifying levels of physical activity that promote healthy body composition is that the relationships between physical activity, energy intake, and body composition can differ dramatically between lean and obese individuals (3,4). This is difficult to resolve using human populations because of the inherent complexity in accurately measuring levels of physical activity and food consumption and controlling for differences in environments and genetic variability.

Given that components of energy balance and body composition are polygenic traits (5), establishment of unique animal models through selective breeding represents a powerful research tool, because the entire biological system that contributes to a phenotype is intrinsically included in the selectively bred outcome. Furthermore, selection for one phenotype can alter correlated traits. For example, selection for high levels of physical activity produces correlated selection responses for increased food intake and decreased body fat (6). Because energy demands for physical activity can be met from food intake and/or stored body fat, we expect that the relationship between physical activity, food intake, and body composition could vary systematically among individuals that are genetically predisposed to be either lean or obese, or to exercise at high or low levels. The purpose of the present study is to compare voluntary wheel running, food consumption, and body composition, and to determine the effects of exercise on body composition, among mice selectively bred for differences in physical activity, growth, and percent body fat.

Voluntary wheel running was the target of a long-term selection experiment in which a base population of Institute of Cancer Research (ICR) mice were bred for high total

[1]Department of Nutrition, University of North Carolina, Chapel Hill, North Carolina, USA; [2]Department of Biology, University of California, Riverside, California, USA; [3]Department of Cell and Molecular Physiology, Carolina Center for Genome Science, University of North Carolina, Chapel Hill, North Carolina, USA; [4]Department of Genetics, Carolina Center for Genome Science, University of North Carolina, Chapel Hill, North Carolina, USA. Correspondence: Daniel Pomp (dpomp@unc.edu)

Received 28 October 2008; accepted 8 February 2009; published online 12 March 2009. doi:10.1038/oby.2009.51

revolutions run on days 5 + 6 of a 6-day exposure to wheels (7). After ~50 generations of selection, mice from the four replicate high runner (HR) lines run approximately three times as many revolutions per day and also exhibit elevated home-cage activity when housed without access to wheels, as compared to mice from four nonselected control lines (8,9). HR lines also have reduced body mass (10,11) and less body fat compared with their ICR control lines (9,10).

Growth and body composition also respond readily to genetic selection. Rapid weight gain from 3 to 6 weeks of age was the target of selection producing M16 mice from a base population of ICR mice (12). M16 mice are heavier, fatter, and hyperphagic compared to their ICR base population at all ages measured (13). More than 20 generations of full-sib mating within this M16 line produced an inbred strain (M16i). A cross between M16 and mice selected for low 6-week weight (L6) (14) served as the base population for selecting mice with a high percentage of body fat while holding body weight constant (HE) (15) and low percentage body fat (LF) (16). Body weights of HE mice do not significantly differ from LF mice, but HE mice have 150% more epididymal fat (17).

In addition to these selection lines, we used two strains as controls. The ICR strain (in this case, specifically the base population for M16 after having undergone long-term random breeding) was chosen because it served as a model for random breeding from a similar base as HR, M16, and M16i, and to a lesser extent the HE and LF selection lines. We also included the C57BL/6J inbred line (B6) because it is often used in mouse biomedical research and anchors most mouse genome databases, including the full genome sequence. Moreover, it has been shown to exhibit low body fat and low metabolic rate under normal feeding conditions (18), as well as relatively low-to-intermediate levels of wheel running (19). As a final group, we included an F1 cross between HR and B6 to facilitate investigation of gene action (20).

We were interested in exploring four basic questions. First, do body weight and body composition vary among strains and sexes? Second, do genetic selection history and sex influence voluntary wheel running? Third, do the means of individual-dependent variables such as body weight and percent body fat after exercise vary by strain and sex, and are there strain by sex interactions effects for these traits? And fourth, is variation for the change in percent body fat after exercise or total food intake during exercise significantly attributable to variation in wheel exercise measures, and is this covariation dependent ont strain × sex subclass?

METHODS AND PROCEDURES

Mouse lines

Table 1 summarizes the genetic selection strains used in this study. The HR strain is one of four replicate lines (University of California, Riverside, designation no. 8) that have been selectively bred for high total revolutions on days 5 + 6 of a 6-day exposure to rat-sized wheels (1.12 m circumference). Full details of the selection procedures are provided elsewhere (7). Male and female HR mice representing 12 different families from generation 44 were shipped from the University of California, Riverside to the Jackson Laboratories for rederivation. Specific-pathogen-free mice representing 11 of these families were then shipped to the University of North Carolina (UNC)–Chapel Hill to establish an HR breeding colony. Generations 1 and 2 from the UNC HR line were used for this experiment. HR × B6 F1 mice were generated by crossing four HR females and two B6 males (Jackson Laboratories, Bar Harbor, ME).

M16 mice were derived from an outbred ICR mouse population by selective breeding for high 3- to 6-week weight gain for 27 generations (12). One family of M16 mice was inbred through repeated full-sib mating for 18 generations to produce the M16i strain. The LF strain was derived from a base population of a cross between M16 and L6 (the L6 strain was derived from a base population originating from a four-way cross of inbred lines (A/Jax, Balb/c, DBA/2Jax, and AKR), which were subsequently bred for small 6-week body weight (14). Selection of LF mice was based on low mass of the right epididymal fat pad as a percentage of body weight, because it is highly correlated with total fat percentage in adult mice (21). Two replicate control lines from the LF experiment were reciprocally crossed and randomly mated for two generations. From this base population, HE mice were created through restricted index selection for high 12-week right epididymal fat pad mass while holding body weight constant (15). Randomly bred ICR control mice from the population used to create the M16 strain were maintained throughout the M16 selection experiments.

Mating pairs of specific-pathogen-free M16, M16i, LF, HE, and ICR mice were transported from North Carolina State University and were used to establish new breeding colonies at UNC. The first and third generations were used for this experiment. For M16i, the second UNC generation was used. For LF, HE, and ICR the second, third, and second UNC generations were used, respectively. For all strains, only first litter offsprings were used in the experiments, and all litters were culled to 7–10 pups at birth.

Table 1 Genetic selection history of strains

Mouse strain	Genetic selection trait	Genetic background	Ref.
HR	High total wheel revolutions run on days 5+6 of a 6-day exposure to wheels	Harlan Sprague Dawley ICR	7
M16	3-6 week high-growth (resulting in obesity)	ICR	12
ICR	Randomly selected with same population parameters as M16	ICR	12
M16i	Inbred by full-sib mating of M16	M16	13
LF	Low mass of right epididymal fat pad as a percentage of body weight	M16 × L6 cross, where L6 strain was bred for small 6-week body weight from a base population originating from a four-way cross of inbred lines (A/Jax, Balb/c, DBA/2Jax, and AKR)	14,16
HE	Restricted index selection for high 12-week right epididymal fat pad mass while holding body weight constant	Base population derived from a cross between two replicate control lines from the LF experiment followed by two generations of random mating	15

Husbandry procedures and experimental design

All mice were housed in standard cages on a 12:12 h light/dark cycle and provided *ad libitum* access to feed and water. Mice were fed Prolab RMH 2000 (Lab Diet: protein 22% of calories, fat 23%, carbohydrates 55%, metabolizable energy 3.52 kcal/g) during the breeding period until the offspring were weaned. Upon weaning, mice were fed Prolab Isopro RMH 3000 (Lab Diet: protein 26%, fat 14%, carbohydrates 60%, metabolizable energy 3.20 kcal/g) through the experimental period. All procedures were conducted in accordance with NIH guidelines for the care and use of experimental animals and based on approved protocols from the Institutional Animal Care and Use Committee of UNC–Chapel Hill.

At 8 weeks of age, body weight and body composition (MRI) were measured before and after 6 days of free access to running wheels for ~15 mice per strain and sex. Total food intake was measured during this 6-day period. Details of methods are provided below.

Wheel-running measurement

Running wheels (model 80850, Lafayette Instrument; circumference = 1.12) were attached to individual high-temperature polycarbonate standard housing cages (11.5 × 7.5 × 5 inch) via 2.5 inch poly(vinyl chloride) tube (diameter 2 inch) that permitted free access. Six sensors spaced 60° apart on the outer perimeter counted revolutions within 1/6 of a revolution using an Activity Wheel Counter (model 86061, Lafayette Instrument, Lafayette, IN) and Running Wheel Activity Software (AWM V9.2, Lafayette Instrument, Lafayette, IN). Our protocol utilized 1-min download intervals over 24 h for distance (cumulative meters), average speed (m/min), maximum speed (fastest speed (m/min) recorded during any 1-min interval within a 24-h period), and minutes (cumulative number of 1-min intervals in which there was at least one wheel revolution recorded).

Body composition

Body composition was measured using an EchoMRI-100 quantitative magnetic resonance whole body composition analyzer (Echo Medical Systems, Houston, TX). The MRI produced output for fat, lean, and water weights in grams. Body weight was measured in grams just prior to MRI.

Table 2 Least-squares means ± s.e. for body weight and body composition

			Body mass (g)		%Fat			%Lean		
Sex	Strain	*n*	In	Out	In	Out	Change	In	Out	Change
♀		130	29.50 ± 0.28	28.08 ± 0.23	17.31 ± 0.32	11.66 ± 0.26	−31.32 ± 1.23	76.58 ± 0.31	80.75 ± 0.25	5.91 ± 0.35
♂		113	36.18 ± 0.30	35.39 ± 0.24	13.26 ± 0.34	8.90 ± 0.28	−29.69 ± 1.30	78.95 ± 0.33	82.21 ± 0.27	4.33 ± 0.37
	HR	35	27.02 ± 0.55^{a}	27.55 ± 0.46^{a}	8.85 ± 0.63^{a}	6.13 ± 0.52^{a}	−31.56 ± 2.46^{a}	84.04 ± 0.62^{a}	84.77 ± 0.5.0^{a}	0.80 ± 0.70^{a}
	F1	33	24.25 ± 0.59^{a}	24.06 ± 0.48^{b}	10.16 ± 0.67^{a}	7.27 ± 0.55^{a,b}	−27.23 ± 2.57^{a,b}	81.84 ± 0.66^{a}	83.39 ± 0.53^{a,b}	2.10 ± 0.74^{a,b}
	B6	26	20.52 ± 0.62^{b}	20.54 ± 0.51^{c}	9.40 ± 0.70^{a,b}	7.72 ± 0.58$^{a–c}$	−13.81 ± 2.70^{c}	83.19 ± 0.69^{a,b}	84.76 ± 0.55$^{a–c}$	1.90 ± 0.78$^{a–c}$
	M16	36	48.05 ± 0.54^{c}	45.76 ± 0.45^{d}	21.50 ± 0.62^{c}	15.65 ± 0.51^{d}	−25.52 ± 2.39$^{a–d}$	72.27 ± 0.61^{c}	76.60 ± 0.49^{d}	6.51 ± 0.69^{d}
	M16i	27	48.00 ± 0.61^{c,d}	45.83 ± 0.50^{d}	17.06 ± 0.69^{d}	12.94 ± 0.57^{d,e}	−21.81 ± 2.67$^{a–e}$	75.69 ± 0.68^{d}	78.83 ± 0.55^{d,e}	4.47 ± 0.77$^{a–e}$
	HE	30	27.87 ± 0.57^{a,e}	26.22 ± 0.47^{a,b,f}	25.58 ± 0.65^{e}	15.50 ± 0.55$^{d–f}$	−39.97 ± 2.51^{a,b,f}	69.23 ± 0.64^{c,e}	76.80 ± 0.51$^{d–f}$	11.15 ± 0.72^{f}
	LF	28	33.55 ± 0.59^{f}	31.07 ± 0.49^{g}	15.51 ± 0.67^{d,f}	8.20 ± 0.55$^{a–c}$	−46.57 ± 2.59^{f,g}	78.17 ± 0.66^{d,f}	84.35 ± 0.53$^{a–c}$	8.06 ± 0.75$^{d–g}$
	ICR	28	33.49 ± 0.59^{f}	32.83 ± 0.49^{g}	14.23 ± 0.67^{d,f}	8.83 ± 0.56$^{a–c}$	−37.61 ± 2.60^{a,b,d,f,g}	77.76 ± 0.66^{d,f}	82.37 ± 0.53$^{a–c}$	5.96 ± 0.75$^{b–e,g}$
♀	HR	20	24.10 ± 0.72	24.75 ± 0.59	9.00 ± 0.81	6.43 ± 0.67	−31.08 ± 3.21	84.63 ± 0.80	84.93 ± 0.64	0.29 ± 0.90
♀	F1	17	21.61 ± 0.79	21.69 ± 0.65	10.60 ± 0.89	8.09 ± 0.74	−23.79 ± 3.46	82.17 ± 0.88	82.95 ± 0.71	1.11 ± 0.99
♀	B6	13	18.51 ± 0.87	18.74 ± 0.71	11.39 ± 0.98	8.94 ± 0.81	−18.92 ± 3.81	82.20 ± 0.97	84.22 ± 0.78	2.52 ± 1.09
♀	M16	23	42.81 ± 0.66	39.75 ± 0.55	27.03 ± 0.75	18.84 ± 0.62	−31.50 ± 2.91	67.81 ± 0.74	74.16 ± 0.60	9.60 ± 0.84
♀	M16i	14	44.93 ± 0.84	41.22 ± 0.69	20.72 ± 0.95	15.90 ± 0.79	−21.09 ± 3.68	72.86 ± 0.94	76.50 ± 0.76	5.35 ± 1.06
♀	HE	15	25.16 ± 0.81	23.42 ± 0.67	28.68 ± 0.92	17.05 ± 0.76	−41.34 ± 3.54	66.77 ± 0.90	75.77 ± 0.73	13.69 ± 1.02
♀	LF	14	28.36 ± 0.84	26.10 ± 0.69	15.01 ± 0.95	8.26 ± 0.78	−43.89 ± 3.67	79.42 ± 0.93	85.35 ± 0.75	7.73 ± 1.05
♀	ICR	14	30.53 ± 0.84	29.00 ± 0.69	16.07 ± 0.95	9.78 ± 0.79	−38.98 ± 3.68	76.78 ± 0.94	82.13 ± 0.75	7.01 ± 1.06
♂	HR	15	29.93 ± 0.82	30.35 ± 0.68	8.69 ± 0.93	5.83 ± 0.79	−32.04 ± 3.60	83.46 ± 0.92	84.61 ± 0.74	1.31 ± 1.03
♂	F1	16	26.88 ± 0.81	26.44 ± 0.67	9.73 ± 0.92	6.44 ± 0.76	−30.67 ± 3.57	81.50 ± 0.91	83.84 ± 0.73	3.10 ± 1.03
♂	B6	13	22.52 ± 0.87	22.35 ± 0.72	7.41 ± 0.99	6.50 ± 0.82	−8.69 ± 3.83	84.17 ± 0.97	85.30 ± 0.79	1.29 ± 1.10
♂	M16	13	53.28 ± 0.89	51.77 ± 0.74	15.96 ± 1.01	12.47 ± 0.84	−19.53 ± 3.92	76.61 ± 1.00	79.04 ± 0.80	3.42 ± 1.13
♂	M16i	13	51.08 ± 0.87	50.45 ± 0.72	13.40 ± 0.99	9.98 ± 0.82	−22.53 ± 3.82	78.51 ± 0.97	81.16 ± 0.78	3.59 ± 1.10
♂	HE	15	30.57 ± 0.81	29.04 ± 0.67	22.48 ± 0.92	13.95 ± 0.76	−38.59 ± 3.54	71.68 ± 0.90	77.83 ± 0.73	8.62 ± 1.02
♂	LF	14	38.74 ± 0.84	36.04 ± 0.69	16.01 ± 0.95	8.13 ± 0.78	−49.25 ± 3.67	76.92 ± 0.93	83.34 ± 0.75	8.40 ± 1.05
♂	ICR	14	36.46 ± 0.84	36.66 ± 0.69	12.38 ± 0.95	7.89 ± 0.78	−36.24 ± 3.67	78.74 ± 0.94	82.60 ± 0.75	4.92 ± 1.05

Body mass, fat mass, and lean mass were measured in grams just prior and immediately after a 6-day wheel-running trial. Percent body fat (and lean) were calculated as (fat mass/body mass) × 100. Change in percent body fat (and lean) were calculated as $((\%fat_{out}-\%fat_{in})/\%fat_{in}) \times 100$. Strain least-squares means (pooled across sex) not sharing a common superscript are different (at least $P < 0.05$). Symbols ♀ and ♂ denote female and male genders, respectively.
B6, C57BL/6J inbred strain; F1, B6XHR; HE, high epididymal fat pad mass with constant body weight; HR, high wheel running; ICR, Institute of Cancer Research outbred strain; LF, Low epididymal fat pad mass as percentage of body weight; M16, rapid weight gain; M16i, inbred line derived from M16.

Table 3 Least-squares means ± s.e. for wheel-running traits

Sex	Strain	Distance	Average speed	Maximum speed	Minutes
♀		10,473.87 ± 257.76	18.84 ± 0.34	32.99 ± 0.42	550.64 ± 8.91
♂		8,428.82 ± 261.59	17.71 ± 0.35	31.73 ± 0.43	452.56 ± 9.04
	HR	14,295.84 ± 492.91[a]	23.90 ± 0.66[a]	42.02 ± 0.81[a]	604.76 ± 17.04[a]
	F1	13,455.11 ± 519.46[a]	25.18 ± 0.69[a]	41.36 ± 0.85[a]	523.81 ± 17.95[a]
	B6	7,241.94 ± 522.91[b]	14.21 ± 0.70[b]	28.50 ± 0.86[b]	496.50 ± 18.07[b]
	M16	7,674.21 ± 474.52[b,c]	17.26 ± 0.63[b,c]	29.12 ± 0.78[b,c]	425.28 ± 16.40[b,c]
	M16i	7,325.27 ± 513.12[b–d]	17.02 ± 0.69[b–d]	30.70 ± 0.84[b–d]	407.78 ± 17.73[b–d]
	HE	6,996.52 ± 477.87[b–e]	14.67 ± 0.64[b–e]	26.24 ± 0.78[b,c,e]	468.30 ± 16.52[b–e]
	LF	8,294.27 ± 496.09[b–f]	16.30 ± 0.66[b–f]	29.34 ± 0.81[b–f]	505.07 ± 17.14[b,c,e,f]
	ICR	10,327.60 ± 499.47[f]	17.69 ± 0.67[b–f]	31.56 ± 0.82[b–d,f]	581.29 ± 17.26[a,b,f]
♀	HR	15,064.73 ± 645.46	23.91± 0.86	42.84 ± 1.06	644.66 ± 22.31
♀	F1	14,198.80 ± 700.70	25.81± 0.94	42.24 ± 1.15	547.41 ± 24.22
♀	B6	7,851.85 ± 759.06	14.64 ± 1.01	29.05 ± 1.24	529.91 ± 26.23
♀	M16	9,424.95 ± 595.20	18.86 ± 0.80	31.33 ± 0.97	493.74 ± 20.57
♀	M16i	8,314.89 ± 735.74	18.15 ± 0.98	31.32 ± 1.20	448.42 ± 25.43
♀	HE	7,666.75 ± 703.64	14.80 ± 0.94	25.96 ± 1.15	513.79 ± 24.32
♀	LF	9,408.74 ± 727.81	16.16 ± 0.97	28.88 ± 1.19	584.14 ± 25.15
♀	ICR	11,860.24 ± 732.39	18.41 ± 0.98	32.27 ± 1.20	643.04 ± 25.31
♂	HR	13,526.95 ± 722.77	23.90 ± 0.97	41.21 ± 1.18	564.86 ± 24.98
♂	F1	12,711.42 ± 727.36	24.54 ± 0.97	40.47 ± 1.19	500.22 ± 25.14
♂	B6	6,632.03 ± 765.59	13.78 ± 1.02	27.95 ± 1.25	463.08 ± 26.46
♂	M16	5,923.46 ± 775.15	15.65 ± 1.04	26.92 ± 1.27	356.82 ± 26.79
♂	M16i	6,335.64 ± 762.36	15.88 ±1.02	30.09 ± 1.25	367.13 ± 26.35
♂	HE	6,326.29 ± 702.50	14.48 ± 0.94	26.51 ± 1.15	422.80 ± 24.28
♂	LF	7,179.81 ± 728.19	16.43 ± 0.97	29.81 ± 1.19	425.99 ± 25.17
♂	ICR	8,794.95 ± 731.71	16.98 ± 0.98	30.84 ± 1.20	519.54 ± 25.29

Results are presented as the average of wheel trial days 5 + 6 for wheel-running distance (m), average speed (m/min), maximum speed (m/min), minutes (number of 1-min intervals in which a wheel revolution was recorded). Strain least-squares means (pooled across sex) not sharing a common superscript are different (at least $P < 0.05$). Symbols ♀ and ♂ denote female and male genders, respectively.
B6, C57BL/6J inbred strain; F1, B6XHR; HE, high epididymal fat pad mass with constant body weight; HR, high wheel running; ICR, Institute of Cancer Research outbred strain; LF, Low epididymal fat pad mass as percentage of body weight; M16, rapid weight gain; M16i, inbred line derived from M16.

Food consumption

Mice were fed from standard wire-top food hoppers. Potential variation in individual food wastage (22) was minimized by collection and weighing of food found in the cage bedding.

Statistical analyses

All traits were analyzed by analysis of covariance or regression using SAS Procedure GLM (SAS, Cary, NC). Main effects for all analyses were strain, sex, and the strain by sex interaction. Age was included as a covariate, although it varied only slightly around 8 weeks. For analysis of wheel traits, we calculated the average of values of days 5 and 6, because this is the criterion for which HR mice were selectively bred (7). Analyses of the running traits included wheel to account for possible effects of wheel-to-wheel variation in position or rotational resistance. Some traits were $\log_{10}$ transformed to improve normality of residuals.

RESULTS

Least-squares means for body mass and body composition measures taken immediately before mice went into the wheels are presented in Table 2. For log body mass there was a significant difference among the strains ($P < 0.0001$), males weighed more than females ($P < 0.0001$), and there was a significant sex by strain interaction ($P < 0.05$). M16 and M16i strains weighed the most, followed by LF and ICR mice, HE and HR, then F1, and finally B6, which was the lightest strain. Percent body fat (fat mass/body mass) × 100 prior to the start of the wheel trial (percent fat) showed a significant effect of sex, strain, and sex by strain interaction (all $P < 0.0001$). HE mice had the highest percent body fat (25.58%) followed by M16, M16i, LF, ICR, F1, B6, and HR (8.85%). Percent lean (lean mass/body mass) × 100 also showed significant effects of sex, strain, and strain by sex (all $P < 0.0001$).

Wheel-running trait least-squares means for sex, strains, and strains separately by sex are presented in the Table 3. For total distance run, minutes, average, and maximum running speed, analyses of covariance indicated highly significant differences among strains (all $P < 0.0001$) and between sexes ($P < 0.0001$, $P < 0.0001$, $P = 0.026$, $P = 0.043$, respectively), with no

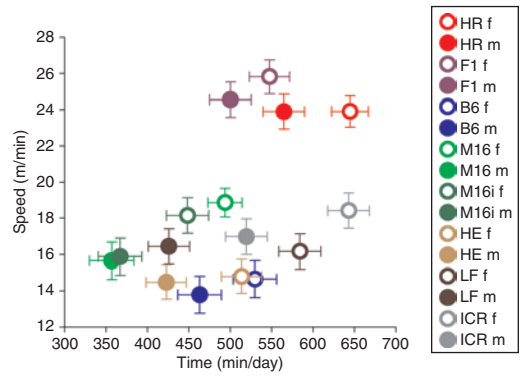

Figure 1 Wheel speed vs. minutes run per day. Sex and strain least-squares means and standard error bars for average number of minutes per day plotted in relation to average wheel speed. Values represent averages of days 5 and 6 of a 6-day exposure to wheels.

strain × sex interaction. Averaged across strains, females ran 24% more revolutions/day, 22% more min/day, and at speeds that were 6.4% (average) and 4.0% (maximum) faster than males. The average running speed was highest for mice from the HR line and in the F1 of HR × B6 (**Figure 1**). The number of minutes run per day was also near the highest for these mice. As a result, the total daily running distance was greatest in the HR and F1 groups, which were statistically indistinguishable from each other. Selection for any traits related to body size and/or composition was related to a decrease in running distance, as seen by comparing M16, M16i, HE, and LF against ICR. This was primarily manifested by mice spending less time exercising, as opposed to slower running speeds (**Figure 1**).

In analysis of covariance controlling for age and average running distance on days 5 and 6, log food intake/gram body mass showed significant effects for strain ($P < 0.0001$) and a strain by sex interaction ($P < 0.0001$) (**Table 4**). Averaging over the sexes, least-squares means (adjusted for multiple comparisons (Scheffe)) showed that HE mice consumed significantly less food than all other strains except M16 (all $P < 0.0001$, except for M16i $P = 0.0177$). The HR strain consumed significantly more food than the other strains except for F1 and B6 mice (all $P < 0.0001$, except for ICR $P = 0.0013$). Overall, adjusted log food intake/gram body mass decreased in the order of HR, F1, B6, ICR, LF, M16i, M16, and HE. Similar results were obtained in a log food intake/gram body mass analysis that did not control for average running distance on days 5 and 6.

Least-squares means for body mass and body composition measures taken immediately after the 6-day wheel trial are presented in **Table 2**. Following the 6-day wheel trial, the percent change in body mass ((body $mass_{out}$–body $mass_{in}$)/body $mass_{in}$) × 100 differed by sex ($P = 0.01$), strain ($P < 0.0001$), and strain by sex ($P < 0.0001$). The percent change in body mass loss was greatest in LF, M16, and M16i mice and least in F1 mice, whereas HR and B6 mice tended to gain body mass. The change ((out–in)/in) × 100 in percent fat during the 6-day wheel trial showed significant effects for sex, strain, and sex by strain (all $P \leq 0.001$), while the change ((out–in)/in) × 100 in percent lean during the 6-day wheel trial also showed a

Table 4 Means and 95% confidence intervals for total food intake per gram body mass

Sex	Strain	Food intake	95% Confidence interval
♀		1.27	1.23–1.32
♂		1.22	1.18–1.27
	HR	1.78[a]	1.65–1.91
	F1	1.70[a,b]	1.57–1.83
	B6	1.44[b,c]	1.33–1.56
	M16	0.84[d]	0.79–0.91
	M16i	1.06[e]	0.98–1.15
	HE	0.88[d]	0.82–0.95
	LF	1.24[c,e,f]	1.15–1.34
	ICR	1.38[c,f]	1.28–1.49
♀	HR	1.84	1.68–2.02
♀	F1	1.86	1.68–2.07
♀	B6	1.54	1.38–1.72
♀	M16	0.77	0.71–0.84
♀	M16i	0.98	0.88–1.10
♀	HE	0.87	0.79–0.97
♀	LF	1.37	1.23–1.53
♀	ICR	1.42	1.28–1.59
♂	HR	1.71	1.54–1.90
♂	F1	1.54	1.39–1.72
♂	B6	1.34	1.20–1.50
♂	M16	0.92	0.82–1.04
♂	M16i	1.14	1.02–1.28
♂	HE	0.88	0.80–0.98
♂	LF	1.11	1.00–1.24
♂	ICR	1.34	1.20–1.50

Food intake is presented as back-transformed values of least-squares means for log transformed total food-intake per gram body mass during the 6-day wheel-running trial. Strain means (pooled across sex) not sharing a common superscript are different (at least $P < 0.05$). Symbols ♀ and ♂ denote female and male genders, respectively.
B6, C57BL/6J inbred strain; F1, B6XHR; HE, high epididymal fat pad mass with constant body weight; HR, high wheel running; ICR, Institute of Cancer Research outbred strain; LF, Low epididymal fat pad mass as percentage of body weight; M16, rapid weight gain; M16i, inbred line derived from M16.

significant effect for strain ($P < 0.01$), strain ($P < 0.0001$), and sex by strain ($P = 0.001$).

Finally, we examined whether the effect of voluntary exercise on changes in percent body fat and log food intake/gram body mass differed among the 16 unique populations of sex and strain by using regression to test the null hypothesis that all slopes are equal. This null hypothesis was rejected for the effects of average and maximum speeds on change in percent body fat ($P \leq 0.01$). The effect of exercise on relative food consumption was not significantly different across the 16 subpopulations for any of the wheel-running variables. We then calculated P values of the 16 individual slope estimates to determine whether the change in percent body fat or log food intake/gram body mass was significantly attributable to any of the four wheel-running traits. As shown in **Table 5**, only HR

Table 5 Effect of wheel running on the change in percent body fat by strain and sex

		Distance run		Average speed		Maximum speed		Minutes in wheel	
Sex	Strain	Slope	*P*	Slope	*P*	Slope	*P*	Slope	*P*
♀	HR	7.23E-04	0.4937	2.32	**0.0009**	2.10	**0.0009**	–0.14	**0.0003**
♀	F1	3.62E-04	0.7105	0.23	0.7644	0.39	0.4830	0.00	0.9409
♀	B6	–3.14E-03	0.2009	0.20	0.9314	–0.15	0.9077	–0.06	0.1642
♀	M16	–2.32E-04	0.8250	–0.32	0.7622	–0.58	0.4603	–0.01	0.6243
♀	M16i	–6.60E-04	0.6977	0.51	0.6670	0.96	0.5533	–0.05	0.2905
♀	HE	–2.95E-03	0.1189	–2.37	0.1248	–0.89	0.5583	–0.05	0.1998
♀	LF	6.97E-04	0.7322	–0.13	0.9485	0.57	0.6795	0.02	0.6929
♀	ICR	1.11E-03	0.6443	1.18	0.5706	1.94	0.3731	–0.01	0.9252
♂	HR	–3.18E-04	0.8089	1.61	0.0975	1.98	**0.0214**	–0.14	**0.0040**
♂	F1	–3.10E-04	0.7496	0.68	0.4229	1.06	0.0765	–0.03	0.4110
♂	B6	–1.56E-03	0.3750	–0.52	0.7002	0.09	0.9464	–0.05	0.3113
♂	M16	–2.96E-03	**0.0342**	–2.40	**0.0179**	–1.46	**0.0496**	–0.07	**0.0374**
♂	M16i	–6.70E-04	0.5742	–0.89	0.3048	–0.60	0.2959	–0.01	0.6616
♂	HE	–4.04E-03	**0.0212**	–2.76	**0.0254**	–2.25	**0.0354**	–0.11	**0.0156**
♂	LF	–5.61E-04	0.8211	–0.58	0.6983	–0.53	0.6286	–0.01	0.8256
♂	ICR	–2.17E-03	0.2630	–1.11	0.3597	–0.31	0.7063	–0.01	0.8457

Wheel-running traits were regressed onto change in percent body fat and the resulting slope estimates were calculated to determine the effects of wheel running on changes in percent body fat. The regression slope estimates the average change in percent fat as running distance (or speed or time in wheels) increases. Boldface *P* values denote instances where the regression slopes indicate a significant increase or decrease in percent body fat as a function of the specific wheel-running trait.
B6, C57BL/6J inbred strain; F1, B6XHR; HE, high epididymal fat pad mass with constant body weight; HR, high wheel running; ICR, Institute of Cancer Research outbred strain; LF, Low epididymal fat pad mass as percentage of body weight; M16, rapid weight gain; M16i, inbred line derived from M16.

mice and HE and M16 males showed significant relationships between a given running trait and change in percent fat loss. Moreover, in HR mice the slopes for the relationships between average speed and change in percent fat loss were strongly positive, whereas for HE and M16 mice they were strongly negative. In other words, as HR mice ran faster, they tended to lose less percent body fat, whereas as HE and M16 males ran faster, they tended to lose more percent body fat. Log food intake/gram body mass was not significantly predicted by any of the four wheel-running variables among any of the 16 subpopulations of strain and sex.

DISCUSSION

This study was designed to explore four questions. First, significant strain, sex, and strain by sex interaction effects were found in body mass, percent body fat and percent lean prior to exercise. Second, we also found significant strain and sex differences in all the wheel-running traits examined. Third, we found that changes in body mass and body composition that occurred following exercise also varied significantly by sex and strain. Finally, we found that the effects of average running speed and maximum speed on the change in percent body fat were significantly dependent upon strain and sex. For example, running speed appeared to have the opposite effect on change in percent body fat in HR mice compared to HE and M16 males. As HR mice ran faster, they lost less percent body fat, whereas HE and M16 males lost more percent fat. While this interaction between genetic background and the relationship between running speed and body composition is provocative, there are scale effects that need to be considered. While M16 and HE mice began the exercise period with large fat stores, HR is a lean line and thus had much less adipose to lose.

One likely factor involved in the etiology of obesity is an inability to effectively oxidize lipids (23). It has long been known that mild- to moderate-intensity exercise increases fatty acid oxidation (24), and there is widespread evidence that exercise is a critical determinant of energy substrate utilization (25–27). In humans, as exercise intensity increases, carbohydrate utilization increases curvilinearly, whereas fatty acid utilization peaks usually around 63% VO_{2max}, and then decreases as exercise intensity increases (28). Given that HR mice run faster (see also ref. 29) and eat more than the other strains (except F1s), it is likely that as HR run faster, they use an increasingly greater percentage of carbohydrates as their energy substrate for running. In light of the extreme levels of wheel running and food intake observed in HR mice, they appear to resemble highly trained human athletes (see ref. 30 and references therein). This view is supported by evidence that during voluntary wheel running, HR mice exhibit a higher voluntary VO_{2max} than controls (31). On the other hand, the M16 strain in particular resembles human obesity and type 2 diabetes (13), and like humans, show a dose–response relationship between exercise and fat loss (32).

HR × B6 F1 mice running distance is significantly greater than B6 mice and comparable to the HR parental strain, which suggests that HR alleles associated with their high wheel-

running selection trait act in a dominant manner. A previous study of the F1 between outbred ICR (the base population for HR) and wild house mice (captured in nature), which ran ~70% more than ICR, also indicated net dominance in the direction of high wheel running (33). From a more general perspective, Bruell (34,35) argues that the demonstrated heterotic inheritance ("hybrid vigor") of wheel-running behavior (indicating significant dominance genetic variance) suggests that wheel running is a selectively important trait. In nature, of course, it would not be wheel running *per se* that is selected, but rather some behavioral (or physiological) trait or traits with which wheel running is closely associated at the genetic level. We have recently found strong parent-of-origin effects on wheel-running traits in a reciprocal HR × B6 cross, whereby mice derived from HR F0 females had higher phenotypes than those derived from a B6 F0 female (S.A. Kelly, D.L. Nehrenberg, T. Garland Jr. and D. Pomp, unpublished data). Because the F1 studied here was only derived from crossing HR females and B6 males, it is possible that their data are increased due to this effect.

While the increased exercise levels in HR mice were predictable, we had no prior information regarding correlated effects of selection for body size and composition in the M16 (and M16i), HE and LF strains on wheel running. All of these selection lines exhibited reductions in overall running distance as a result of spending less time in the wheels, relative to the randomly selected ICR line. Reduced exercise in the larger and fatter M16 line was not surprising, as they might be expected to tire more easily. HE is smaller and fatter than LF, but they had relatively similar exercise phenotypes. These lines may have run less than ICR due to differences in genetic background, having had M16 and several inbred lines as part of the base population from which selection was initiated.

To our knowledge only one other study has examined whether selection for body composition (e.g., lean vs. obese) involve correlated changes in exercise activity using mice. Simoncic *et al.* (36) recently compared running wheel activity between mice bidirectionally selected for low (L) and high percentage body fat (F). While L and F running wheel activity was initially similar, by the end of the 40-day wheel trial F mice ran 40% as much as L mice, exhibited significantly less home-cage activity, and ate significantly less food per day than L mice (36). Because there are stark contrasts between our methods and those used by Simoncic *et al.* (36), it is difficult to draw straightforward comparisons. Despite the methodological differences, it is noteworthy that selection for either low percent body fat (L) or high wheel running (HR) appears to exert convergent effects. Selection for lean mice produced high-running mice, and selection for high running produced lean mice. This net selection effect could indicate that high physical activity and lean body composition are genetically correlated. Or perhaps small body size is a prerequisite for high running levels because it avoids the higher energy costs of moving a larger body (37).

Given that this was the first analysis of exercise-induced changes in body composition in most of the strains evaluated, we have not yet examined the potential physiological mechanisms underlying the significant differences found. However, several previous studies in some of these strains provide glimpses into possible underpinnings of how different genetic selection history may have (or have not) changed the way mice respond to exercise. As a few examples of many, HR mice and their control lines do not differ in resting or basal metabolic rate, or respiratory exchange ratio measured under those conditions (38). However, HR have elevated maximal oxygen consumption during forced treadmill exercise (39). HR do not show generally altered muscle fiber-type composition, although some differences in the tibialis anterior muscle have recently been detected (40). M16 and M16i have significantly lower heat loss than ICR, indicating reduced basal metabolic rate as a correlated response to selection, and also have less brown adipose tissue relative to body weight (13). Further studies will be required to understand how these, and other relevant physiological mechanisms, relate to variation in exercise-induced canges in body composition in HR, M16, and the other selection lines used in the present study.

In human studies examining the effect of exercise on fat oxidation it is often found that a large portion of interindividual variation goes largely unexplained (4), even among trained athletes (41). There are considerable individual differences for health-related exercise training responses that appear attributable to genetic variation, but no robustly significant genes have been found for exercise response phenotypes in human gene association studies (see ref. 42 and references therein).We found the relationship between exercise and change in body fat to be complex, because this relationship depended on genetic selection history. Three mouse strains selectively bred for exercise or body composition showed a significant relationship between exercise and change in body fat, but the common inbred line B6 and outbred strain ICR did not. Voluntary wheel-running activity (43) and effects of exercise on body composition (44) are all traits that have a complex genetic architecture. But because the effects of exercise on body composition depend on genetic background, and variation for change in percent body fat is not always directly attributable to variation in exercise measures, it is important to carefully consider genetic background and/or selection history when using mice to model effects of exercise on body composition. By extension, similar consideration may be required when modeling effects of exercise on other complex, polygenic traits. Given that nearly all mice will run when provided access to wheels, our finding of significant genetic variation in exercise-induced changes to body composition may be applicable to the subpopulation of humans who voluntarily exercise as opposed to those who remain sedentary.

ACKNOWLEDGMENTS

This work was partially supported by NIDDK grant DK076050 to DP and NSF grant IOB-0543429 to TG. We also thank the pilot funding of the Interdisciplinary Obesity Center (UNC) and NIH 1 P20 RR020649 for their support. D.L.N. was partially supported by the UNC Curriculum in Toxicology Training Grant T32 ES007126, while D.E.-S. was partially supported by a Seeding Postdoctoral Innovators in Science and Education (SPIRE) fellowship from NIGMS (grant GM00678). Phenotypes were collected using the Animal Metabolism Phenotyping core facility within UNC's Clinical Nutrition Research Center (funded by NIDDK grant DK56350). We thank

Chris Wiesen at UNC's Odum Institute of Social Science for data analysis consultation.

DISCLOSURE

The authors declared no conflict of interest.

REFERENCES

1. Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. *Cell* 2001;104:531–543.
2. Jakicic JM, Otto AD. Treatment and prevention of obesity: what is the role of exercise? *Nutr Rev* 2006;64:S57–S61.
3. Melzer K, Kayser B, Saris WH, Pichard C. Effects of physical activity on food intake. *Clin Nutr* 2005;24:885–895.
4. Venables MC, Achten J, Jeukendrup AE. Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *J Appl Physiol* 2005;98:160–167.
5. Pomp D. Genetic dissection of obesity in polygenic animal models. *Behav Genet* 1997;27:285–306.
6. Swallow JG, Koteja P, Carter PA, Garland T Jr. Food consumption and body composition in mice selected for high wheel-running activity. *J Comp Physiol [B]* 2001;171:651–659.
7. Swallow JG, Carter PA, Garland T Jr. Artificial selection for increased wheel-running behavior in house mice. *Behav Genet* 1998;28:227–237.
8. Malisch JL, Breuner CW, Gomes FR, Chappell MA, Garland T Jr. Circadian pattern of total and free corticosterone concentrations, corticosteroid-binding globulin, and physical activity in mice selectively bred for high voluntary wheel-running behavior. *Gen Comp Endocrinol* 2008;156:210–217.
9. Vaanholt LM, Jonas I, Doornbos M *et al*. Metabolic and behavioral responses to high-fat feeding in mice selectively bred for high wheel-running activity. *Int J Obes (Lond)* 2008;32:1566–1575.
10. Swallow JG, Koteja P, Carter PA, Garland T. Artificial selection for increased wheel-running-activity in house mice results in decreased body mass at maturity. *J Exp Biol* 1999;202:2513–2520.
11. Dumke CL, Rhodes JS, Garland T Jr *et al*. Genetic selection of mice for high voluntary wheel running: effect on skeletal muscle glucose uptake. *J Appl Physiol* 2001;91:1289–1297.
12. Hanrahan JP, Eisen EJ, Legates JE. Effects of population size and selection intensity on short-term responses to selection for post-weaning gain in mice. *Genetics* 1973;73:513–530.
13. Allan MF, Eisen EJ, Pomp D. The M16 mouse: an outbred animal model of early onset polygenic obesity and diabesity. *Obes Res* 2004;12:1397–1407.
14. Legates JE. Direct and correlated responses to selection in mice. *In: Bogart R (ed). Genetic Lectures*, Oregon State University Press: Corvallis, Oregon, 1969, *pp* 149–165.
15. Eisen EJ. Restricted index selection in mice designed to change body fat without changing body weight:directed responses. *Theor Appl Genet* 1992;83:973–980.
16. Eisen EJ. Selection for components related to body composition in mice: direct responses. *Theor Appl Genet* 1987;74:793–801.
17. Fan YK, Croom WJ Jr, Daniel LR *et al*. Selection for body composition does not affect energetic efficiency of jejunal glucose uptake in mice. *J Nutr* 1996;126:2861–2866.
18. Moody DE, Pomp D, Nielsen MK. Variability in metabolic rate, feed intake and fatness among selection and inbred lines of mice. *Genet Res* 1997;70:225–235.
19. Lightfoot JT, Turner MJ, Daves M, Vordermark A, Kleeberger SR. Genetic influence on daily wheel running activity level. *Physiol Genomics* 2004;19:270–276.
20. Hannon RM, Kelly SA, Middleton KM *et al*. Phenotypic effects of the "mini-muscle" allele in a large HR × C57BL/6J mouse backcross. *J Hered* 2008;99:349–354.
21. Eisen EJ, Leatherwood JM. Predicting percent fat in mice. *Growth* 1981;45:100–107.
22. Koteja P, Carter PA, Swallow JG, Garland T Jr. Food wasting by house mice: variation among individuals, families, and genetic lines. *Physiol Behav* 2003;80:375–383.
23. Zurlo F, Lillioja S, Esposito-Del Puente A *et al*. Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. *Am J Physiol* 1990;259:E650–E657.
24. Krogh A, Lindhard J. The relative value of fat and carbohydrate as sources of muscular energy: with appendices on the correlation between standard metabolism and the respiratory quotient during rest and work. *Biochem J* 1920;14:290–363.
25. Achten J, Gleeson M, Jeukendrup AE. Determination of the exercise intensity that elicits maximal fat oxidation. *Med Sci Sports Exerc* 2002;34:92–97.
26. Thompson DL, Townsend KM, Boughey R, Patterson K, Bassett DR Jr. Substrate use during and following moderate- and low-intensity exercise: implications for weight control. *Eur J Appl Physiol Occup Physiol* 1998;78:43–49.
27. Romijn JA, Coyle EF, Sidossis LS *et al*. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol* 1993;265:E380–E391.
28. Achten J, Jeukendrup AE. Optimizing fat oxidation through exercise and diet. *Nutrition* 2004;20:716–727.
29. Girard I, McAleer MW, Rhodes JS, Garland T Jr. Selection for high voluntary wheel-running increases speed and intermittency in house mice (*Mus domesticus*). *J Exp Biol* 2001;204:4311–4320.
30. Guderley H, Joanisse DR, Mokas S, Bilodeau GM, Garland T Jr. Altered fibre types in gastrocnemius muscle of high wheel-running selected mice with mini-muscle phenotypes. *Comp Biochem Physiol B Biochem Mol Biol* 2008;149:490–500.
31. Rezende EL, Chappell MA, Gomes FR, Malisch JL, Garland T Jr. Maximal metabolic rates during voluntary exercise, forced exercise, and cold exposure in house mice selectively bred for high wheel-running. *J Exp Biol* 2005;208:2447–2458.
32. Ohkawara K, Tanaka S, Miyachi M, Ishikawa-Takata K, Tabata I. A dose–response relation between aerobic exercise and visceral fat reduction: systematic review of clinical trials. *Int J Obes (Lond)* 2007;31:1786–1797.
33. Dohm MR, Richardson CS, Garland T Jr. Exercise physiology of wild and random-bred laboratory house mice and their reciprocal hybrids. *Am J Physiol* 1994;267:R1098–R1108.
34. Bruell JH. Heterotic inheritance of wheel running in mice. *J Comp Physiol Psychol* 1964;58:159–163.
35. Bruell JH. Inheritance of behavioral and physiological characters of mice and the problem of heterosis. *Am Zool* 1964;4:125–138.
36. Simoncic M, Horvat S, Stevenson PL *et al*. Divergent physical activity and novel alternative responses to high fat feeding in polygenic fat and lean mice. *Behav Genet* 2008;38:292–300.
37. Taylor CR, Schmidt-Nielsen K, Raab JL. Scaling of energetic cost of running to body size in mammals. *Am J Physiol* 1970;219:1104–1107.
38. Kane SL, Garland T Jr, Carter PA. Basal metabolic rate of aged mice is affected by random genetic drift but not by selective breeding for high early-age locomotor activity or chronic wheel access. *Physiol Biochem Zool* 2008;81:288–300.
39. Rezende EL, Gomes FR, Malisch JL, Chappell MA, Garland T Jr. Maximal oxygen consumption in relation to subordinate traits in lines of house mice selectively bred for high voluntary wheel running. *J Appl Physiol* 2006;101:477–485.
40. Bilodeau GM, Guderley H, Joanisse DR, Garland T Jr. Reduction of type IIb myosin and IIB fibers in tibialis anterior muscle of mini-muscle mice from high-activity lines. *J Exp Zool Part A: Ecol Genet Physiol*, *in press*.
41. Goedecke JH, St Clair, Gibson A, Grobler L *et al*. Determinants of the variability in respiratory exchange ratio at rest and during exercise in trained athletes. *Am J Physiol Endocrinol Metab* 2000;279: E1325–E1334.
42. Rankinen T, Bouchard C. Gene-physical activity interactions: overview of human studies. *Obesity (Silver Spring)* 2008;16(Suppl 3):S47–S50.
43. Lightfoot JT, Turner MJ, Pomp D, Kleeberger SR, Leamy LJ. Quantitative trait loci for physical activity traits in mice. *Physiol Genomics* 2008;32:401–408.
44. Chagnon YC, Rice T, Perusse L *et al*. Genomic scan for genes affecting body composition before and after training in Caucasians from HERITAGE. *J Appl Physiol* 2001;90:1777–1787.