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Phenotypic and evolutionary plasticity of body composition in rats selectively bred for high endurance capacity

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Swallow JG, Wroblewska AK, Waters RP, Renner KJ, Britton SL, Koch LG. Phenotypic and evolutionary plasticity of body composition in rats selectively bred for high endurance capacity. *J Appl Physiol* 109: 778–785, 2010. First published June 17, 2010; doi:10.1152/jappphysiol.01026.2009.—We investigated the effects of genetic selection and prolonged wheel access (8 wk) on food consumption and body composition in lines of rats selected for high and low intrinsic (untrained) endurance running capacity (HCR and LCR, respectively) to test the generality of phenotypic correlations between physical activity levels, aerobic capacity, and body composition. HCR rats ran more minutes per day on activity wheels than LCR rats, supporting the hypothesis that voluntary activity and physiological capacity are genetically correlated (self-induced adaptive plasticity). Both treatments (selection and wheel access) significantly affected food consumption. HCR rats consumed and digested more food than LCR rats. Access to running wheels did not result in changes in overall body mass, but lean body mass increased and percent body fat decreased in both lines. Selection for high endurance capacity resulted in hypertrophy of the heart and kidneys and decreased long intestine length. We found significant phenotypic flexibility in a number of organ masses after wheel running. Specifically, access to running wheels resulted in hypertrophy of the heart, liver, kidney, stomach, and small and large intestines in LCR and HCR rats. The selected line \times wheel access interaction was significantly greater in HCR rats in relative mass for the heart and lung. Compared with LCR rats, HCR rats fortify wheel running with increased food consumption along with greater hypertrophy of key organs for O₂ transport.

wheel-running activity; correlated response; acclimation; organ masses; food consumption

A GROWING NUMBER OF HUMAN studies assume a significant degree of genetic determination for the correlations between physiology, morphology, and behavior related to physical exercise. For example, a collection of international database surveys from 37,051 twin samples proposes that the genetic component for willingness to participate in exercise activities has a median heritability of 62% and may involve genes that associate with physiological level of exercise ability, body composition, and eating behaviors (47). A series of association and linkage studies performed with nuclear families (the HERITAGE Family Study) suggest that the propensity for maximal exercise capacity, physical activity, and storage of visceral fat can be acquired through genotype (2, 39, 40, 42, 46, 54). More recently, the first genome-wide study of habitual exercise activity revealed 37 single-nucleotide polymorphisms,

yet none colocalized with those in genetic regions previously reported as most significant for exercise and physical activity phenotypes (5). While evidence from human studies suggests that a substantial genetic component exists for exercise-related complex traits, the mechanistic factors that explain associations between these traits are difficult to elucidate (38).

In animal models, the genetic relationship between exercise behavior, endurance capacity, and body composition is also unclear. While early studies on outbred mice suggest that treadmill and wheel running may be related to aerobic physiological capacity (6), tests for genetic determination across several different inbred strains of mice have shown no correlation between wheel-running behavior and aerobic capacity/endurance (7, 29) and at least one other test has shown no commonality in trait loci between wheel-running and exercise endurance phenotypes (30). On the other hand, an association between endurance capacity and body composition has been demonstrated in lines of outbred house mice artificially selected over several generations for a high level of voluntary wheel-running activity (36, 49). Similarly, a genetic correlation with increased wheel-running activity and low body mass was reported in heterogeneous rat models selectively bred for intrinsic (inborn) endurance exercise capacity assessed by forced treadmill running (20, 53). The correlation between endurance capacity, body type, and physical activity in selectively bred rodents generalizes to home cage activity as well (31, 38, 41). Recently, Novak et al. (38) extended these ideas from rats to humans and presented evidence for a positive correlation between maximal O₂ consumption ($\dot{V}O_{2max}$) and daily nonexercise activity.

In 2005, Swallow et al. (52) coined the term “self-induced adaptive plasticity” to describe situations, as was apparent with the high-wheel-running-selected mouse lines, in which organisms engage in behaviors that positively feed back on their physiological capacity to engage in that behavior. They speculated that the self-reinforcing phenomenon of beneficial acclimation of physiological traits that support the expression of complex, behavioral-physiological phenotypes might be quite common (as demonstrated in Ref. 38).

The N:NIH out-crossed stock of rats bidirectionally selected for intrinsic endurance capacity (20) provides a mechanistic-based, contrasting model system to test the phenotypic relationships between an inherent level of aerobic capacity, the behavior for increased physical activity, and a series of flexible intermediate phenotypes hypothesized to support and sustain aerobic metabolic function. Previous reports show that rats bred as low-capacity runners (LCR) and high-capacity runners (HCR) differed by 347% for treadmill-running distance (20) and diverged for peripheral traits, e.g., capillary density and

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muscle oxidative enzyme activity (17, 19, 55), as well as central phenotypes, e.g., heart size, cardiac output, and stroke volume (9), that functionally determine aerobic capacity. Because these lines differ dramatically in aerobic capacity, we tested the hypothesis that organ sizes, particularly those involved in O₂ delivery and energy extraction, would concomitantly diverge.

Relatively little attention has been paid to the phenotypic flexibility of visceral organs in response to exercise (see Table 7 in Ref. 3 for a review). The best evidence illustrating how flexible changes in internal organ structure are correlated with improved functional capacity and metabolic function comes from experiments in which rodents were exposed to chronic energy challenges. For example, rodents exposed to prolonged cold exhibit not only hypertrophy of cardiac muscle but also hypertrophy of many internal organs required to support nutrient extraction and utilization, including the gastrointestinal (GI) tract, liver, and kidney (11, 14, 22, 26, 32, 33). Similar patterns are also exhibited in response to increased energy demand from conditions such as lactation and exposure to high altitude (10, 13). Relative to sedentary controls, rodents with access to running wheels increase food consumption to support increases in energy demand (24, 51). Wheel running, therefore, might bring about physiological adaptations in the visceral organs required to support nutrient extraction and utilization.

Exercise training often causes physiological adaptations that are generally reversible and, thus, serve as well-documented examples of phenotypic flexibility. A goal of this study was to compare magnitude and direction of phenotypic flexibility (acclimation response to wheel running) with the magnitude and direction of evolutionary plasticity (selection for increased endurance capacity). Thus, in a two-way design, we studied the effects of genetic selection history (10 generations) and exercise history (8 wk of access to running wheels) on total body mass and composition (percent body fat) as well as on organ mass of the left ventricle, lungs, liver, kidneys, spleen, stomach, and small and large intestines. Food consumption and energy assimilation were also measured to assess the degree to which wheel access increased energy demand.

MATERIALS AND METHODS

All procedures were carried in accordance with the National Research Council *Guide for Care and Use of Laboratory Animals* (34). The following protocol was approved by the Institutional Animal Care and Use Committee of the University of South Dakota.

Study animals and housing. The population of rats used in this study was from an artificial selection experiment for low- and high-endurance running capacity (LCR and HCR, respectively). The development of the rat models is described in detail elsewhere (20). Briefly, bidirectionally selected lines were started from a founder population of 80 male and 88 female N:NIH stock rats based on a measure of intrinsic aerobic treadmill-running capacity. Thirteen families each were set up for a within-family rotational breeding paradigm. This schedule permits <1% inbreeding per generation to maintain heterogeneity within each selected line.

At each generation, young adult (11-wk-old) rats were phenotyped for inherent ability to perform a speed-ramped treadmill-running test. This test was patterned after clinical treadmill tests and provides a reliable estimate of endurance exercise capacity as a means to segregate large populations of rats into LCR and HCR across generations. The protocol consisted of running each rat on a motorized treadmill set at 10 m/min on a 15-degree slope and electrically programmed

increases in speed (1 m every 2 min) until rats reached exhaustion. Rats were tested daily over 5 consecutive days, and the greatest distance run in meters out of the five trials was considered the best estimate of intrinsic exercise capacity (20). The highest-scoring female and male from each of the 13 families were selected as breeders for the next generation of HCR. The same process was used with lowest-scoring females and males to generate LCR.

A group of 20 closely age-matched LCR and HCR female rats were specially bred from former *generation 9* and *10* breeders, phenotyped for intrinsic endurance capacity, and then shipped by air to the University of South Dakota animal facility for study. We used only female rats, because females exhibit a greater response to selection for endurance treadmill capacity (20) than males. Furthermore, in addition to sex differences in the selected character, sex differences in rodents for wheel running are not uncommon, with females running more than males (48). Together, these factors should facilitate detection of a correlated response in activity, body composition, and food consumption.

Rats were housed individually in clear plastic Nalgene cages (43 × 27 × 15 in.) with cedar bedding and wire lids. Food (Teklad Rodent Diet 8604) and water were available ad libitum. An automatic timer was used to maintain a 12:12-h dark-light cycle (dark period from 0900 to 2100). Body mass was measured on *day 6* of every week at the end of the dark period (between 2000 and 2100). During measurement of body mass, each animal's bedding, food, and water were changed. Rats were given 20 days to acclimate to this environment before introduction of the running wheel at, on average, 147 (range 141–157) days of age.

Voluntary wheel running. After the acclimation period, rats from both strains were randomly assigned to a wheel group ($n = 10$ LCR and 10 HCR) or a sedentary group ($n = 10$ LCR and 10 HCR). At this point, animals were weighed (± 0.1 g), and those in the wheel group were given access to a 1.084-m-circumference wheel (Nalgene Activity Wheels, Fisher Scientific, Pittsburgh, PA), which was introduced into the cage at the time of body mass assessment. Sedentary animals did not have access to a wheel and were used as controls to determine the effect of a running wheel on body mass, body composition, and food consumption.

Running wheels were connected to a Mini Mitter system that used a magnetic sensor to record wheel rotations. This system was interfaced with a computer, and data were recorded using VitalView software (Mini Mitter, Bend, OR) at 1-min intervals 24 h/day for 8 wk. Total daily distance was calculated as number of revolutions × wheel circumference. For each animal, time spent running was calculated as the sum of 1-min bins during which any activity was recorded, and running speed was calculated as meters per minute during those active minutes (48). Only wheel-running data from *weeks 5* and *6* (*days 29–42*) that correspond with the rats' food consumption measures are presented here. A detailed microanalysis of these running data, for the entire period of wheel access, has been presented previously (53). Because of malfunctions in data collection and storage associated with three running wheels, running data from two HCR animals and one LCR animal were omitted.

Food consumption. Rates of food consumption and digestion were measured during *weeks 5* and *6* according to the protocols of Swallow et al. (51) with slight modifications. During this period, no bedding or nesting material was provided; instead, metal grids were suspended over the floor on the Nalgene cages. At the beginning of each week, animals were weighed (± 0.1 g) and a weighed portion of food (± 0.01 g; Harlan Teklad Rodent Diet 8604) was placed in the hoppers. Water was provided ad libitum. Samples of the food were taken for measurement of dry mass content. At the end of each week, the cages were changed. All uneaten food, including orts (spilled and leftover food), and feces were collected, segregated manually, and dried at 60°C to a constant mass.

Daily food consumption (DFC, g/day) was calculated as follows: $DFC = [(food\ given \times dry\ mass\ content) - dry\ food\ eaten]/day$.

Daily food digested (DFD, g/day) was calculated as follows: DFD = (dry food eaten – dry feces)/number of days.

Apparent food digestibility (D) was calculated as follows: $D = (DFD/DFC) \times 100$.

Body composition. Between days 42 and 49 of wheel access, female rats were monitored for stages of the estrous cycle by assessment of the cytology of daily vaginal lavages (between 2000 and 2100) with 0.9% saline via an eyedropper. This was done to ensure that all rats were killed during diestrus II to control for hormonal variability, which might impact the current measurements, as well as neuroendocrine measurements, which are presented elsewhere (53).

With use of a randomized design, the animals were weighed (± 0.1 g) and then killed by rapid decapitation between 1000 and 1200 on days 57, 58, 59, and 60. After a midventral incision, the ventricles (trimmed of atria and major blood vessels), liver (with gallbladder removed), lungs, kidneys, and spleen were dissected, blotted, and weighed (± 0.01 g). The GI tract was dissected, trimmed of fat and connective tissue, and separated into three parts: stomach, small intestine, and large intestine. Each section of the GI tract, including contents, was weighed (± 0.01 g), flushed with an isotonic physiological saline solution (0.9% NaCl) to remove gut contents, blotted to remove excess fluid, and reweighed (± 0.01 g). For measurement of intestine length (± 1 mm), the flushed organ section was gently laid along the length of a flat ruler to avoid stretching. The dissected organs, except the brain, and all trimmed tissue were placed inside the carcass. The carcasses were stored in individual tissue bags at -20°C until they were freeze-dried.

Whole animals were lyophilized to a stable mass and then homogenized by blending in liquid nitrogen. From each homogenate, two ~ 1 -g samples were placed in extraction thimbles and stored in a desiccator. Samples were refluxed with petroleum ether for 12 h (51) in a Goldfish apparatus (Labconco) for measurement of fat content. Percent body fat was calculated as percentage of fat in the dry mass from each sample taken [$\% \text{fat} = [\text{extracted fat (g)}/\text{sample (g)}] \times 100$] and then averaged for each animal. Lean body mass was approximated by subtraction of this estimated total body fat from the final body mass.

Statistics. One-way ANOVA was used to compare the effects of selection (HCR vs. LCR) on wheel-running activity. Measures of running activity analyzed in this study include meters run per day, average running speed, and minutes run per day. All running traits were scored as the mean of the 14 days of wheel running during the feeding trials. Two-way ANOVA and analysis of covariance (ANCOVA) were used to compare the effects of selection and wheel access (with vs. without running wheel) with regard to final body mass, body composition, and food consumption. Because different traits scale differently with body mass, we chose to use statistical methods that account for body mass, rather than to report these data using ratios (trait/body mass). Average body mass during the 2 wk of the feeding was used as a covariate in analyses of food consumption. Similarly, body mass at the time the animals were killed was used in analyses of body composition. Statistical tests were performed using the ANOVA function in SYSTAT version 10.2, which allows for fixed and random effects. All values are presented as means \pm SD (ANOVA) or least-squares-adjusted means \pm SD (ANCOVA) in cases where we needed to account for body mass.

RESULTS

Study animals. At 11 wk of age, HCR rats exhibited more than fivefold greater intrinsic (untrained) endurance capacity than LCR rats ($1,062.2 \pm 165.16$ vs. 192.2 ± 50.71 m, $P < 0.001$; see Fig. S1 in Supplemental Material available online at the Journal website). Endurance capacity did not differ between groups within selected lines based on wheel assignment ($P = 0.284$). At the beginning of the study, HCR rats were significantly lighter than LCR rats (165.2 vs. 199.4 g, $P < 0.001$). Body mass did not differ between groups within selected lines based on wheel assignment ($P = 0.095$). This pattern held across all weeks of the study, including the 2 wk of the feeding trial (see below).

Voluntary wheel running. Daily wheel-running activity during the 2 wk of the feeding trial is presented in Table 1. During this period, HCR rats given access to running wheels exhibited a trend toward a greater daily running distance than did LCR animals ($P = 0.058$). HCR rats exhibited greater running duration than LCR rats ($P = 0.019$). However, average running speed did not differ between the two lines ($P = 0.211$).

Food consumption. Based on two-way ANCOVA, whole animal rates of daily food consumption increased with wheel access but were not significantly altered by bidirectional selection for endurance capacity (Table 2). However, because food consumption was positively correlated with body mass and because the selected lines differed significantly in body mass, comparisons between the lines in any aspect of food consumption that do not take body mass into account are suspect. Therefore, to correct for body mass, all subsequent analyses are based on two-way ANCOVAs with body mass as a covariate.

When adjusted for effects of body mass, selected line and wheel access significantly affected daily food consumption. Using the adjusted means from two-way ANCOVA [i.e., mass-corrected value from least-square means (LSMEANS)], we found that, in general, wheel access increased food intake; rats with access to activity wheels ate 5.12 g/day more than rats housed without activity wheels (20.16 vs. 15.04 g/day), representing a 34.0% increase (note that LSMEANS for wheel access values represent an average from LCR and HCR combined for the with-wheel vs. without-wheel groups). While statistically significant, effects of selection for endurance capacity on food consumption were smaller: HCR rats ate 2.71 g/day more than LCR rats (18.96 vs. 16.25 g/day), representing a 16.7% increase (note that LSMEANS for selection values represent an average from with-wheel vs. without-wheel treatment combined for the LCR and HCR groups). Furthermore, the difference in food consumption rates between the active and sedentary animals was greater in the HCR line (7.04 g, representing a 46% increase with wheel access) than in the LCR line (3.21 g, representing a 22% increase with wheel

Table 1. Daily wheel-running activity and its components separated by selected line

Line	n	Distance, m/day	Time, min/day	Speed, m/min
HCR	8	$20,674.1 \pm 8,859.44$	$414.1 \pm 72.07^*$	48.5 ± 48.48
LCR	9	$12,374.1 \pm 2,627.43$	294.9 ± 109.71	38.1 ± 38.11

Values (means \pm SD) are daily averages from weeks 5 and 6 (days 29–42) that correspond with the rats' food consumption measures. HCR, high-capacity runners; LCR, low-capacity runners. $*P < 0.05$.

Table 2. Least-squares-adjusted (i.e., mass-corrected) daily food consumption and digestibility data

Trait	n	Low		High		Significance (P value)			Body mass
		Sedentary	Active	Sedentary	Active	Line	Wheel	Line × wheel access	
Body mass, g	40	247.3 ± 24.87	260.9 ± 24.02	216.1 ± 8.54	219.2 ± 22.79	<0.001	0.252	0.472	
DFC, g/day									
Whole animal	40	15.11 ± 1.876	19.33 ± 1.830	14.60 ± 1.014	21.77 ± 1.735	0.073	<0.001	0.008	
Mass-adjusted	39	14.64 ± 1.395	17.85 ± 1.620	15.44 ± 1.499	22.48 ± 1.458	<0.001	<0.001	<0.001	<0.001
Food digested, g/day	40	11.42 ± 1.072	14.39 ± 1.199	11.75 ± 1.138	16.85 ± 1.110	0.003	<0.001	0.003	0.002
Digestibility	38	77.3 ± 0.98	78.2 ± 1.05	77.0 ± 0.98	75.4 ± 0.95	<0.001	0.258	<0.001	0.560
Orts									
g	40	0.32 ± 0.515	1.32 ± 0.576	0.46 ± 0.547	1.41 ± 0.534	0.594	<0.001	0.996	0.872
%	40	2.5 ± 2.72	7.0 ± 3.04	3.0 ± 2.88	6.2 ± 2.81	0.892	<0.001	0.446	0.440

Values are means ± SD. DFC, daily food consumption. Significance of main effects, selected line × wheel access interaction, and body mass covariate was tested by ANOVA or analysis of covariance (ANCOVA). Effects that are significant at $P < 0.05$ are shown in boldface.

access), as reflected by the significant interaction between selected line and wheel access (Table 2).

Amount of food digested per day showed patterns qualitatively similar to those for daily food consumption. The average value for mass-adjusted digestibility across all treatment groups was 77%. The digestibility coefficient was 1.4% lower in the HCR (76.3%) than in the LCR (77.7%) line (LSMEANS for selection). Although wheel access did significantly affect the digestibility coefficient, the interaction between selected line and wheel access was significant, because access to running wheels had differential effects on digestibility. Digestibility decreased in the HCR lines with wheel access, while it increased in the LCR lines (Table 2).

The amount of mass-adjusted food fragmented into Orts was greater in the groups with access to running wheels. Measured as total mass, the amount of food fragmented more than tripled with wheel access (0.39 vs. 1.37 g), while measurements of food fragmented as a percentage of food eaten more than doubled (2.73 vs. 6.64%) with wheel access (LSMEANS for wheel access). Neither selected line nor body mass had a significant effect on food fragmentation (Table 2).

Body composition. Table 3 presents LSMEANS, standard errors, and significance levels from two-way ANOVAs for total body mass, lean body mass, and percent body fat and two-way ANCOVAs for organ masses and sizes. At the end of

8 wk of wheel access, HCR rats were lighter than LCR rats and wheel access did not significantly influence body mass. However, selected line and wheel access significantly affected percent body fat (i.e., mass-corrected values from LSMEANS for selection and wheel access, respectively); HCR rats had less body fat than LCR rats (19.2 vs. 23.8%), and rats with wheel access had reduced body fat compared with rats without wheel access (18.2 vs. 24.8%). Similarly, selected line and wheel access affected lean body mass. HCR rats had significantly less lean body mass than LCR rats (177.6 vs. 192.4 g), while rats with wheel access had significantly increased lean body mass compared with rats without wheel access (196.5 vs. 173.4 g). The selected line × wheel access interaction approached, but did not reach, significance, indicating that the scope of the change in lean body mass was similar in both selected lines, although it was slightly higher in the LCR line (Table 3).

Rats with access to running wheels had larger mass-adjusted heart, liver, right and left kidney, stomach, small intestine, and large intestine mass, as well as longer small intestine and large intestine, than sedentary rats. Wheel access also resulted in reduced mass-adjusted spleen mass. Selected line effects were more limited. HCR rats had increased mass-adjusted heart and right kidney mass and reduced relative large intestine length compared with LCR rats. In our analysis, interactions between selected line and wheel access can be considered a genotype ×

Table 3. Least-squares-adjusted (i.e., mass-corrected) body composition data

	n	Low		High		Significance (P value)			Body mass
		Sedentary	Active	Sedentary	Active	Line	Wheel	Line × wheel access	
Body mass, g	40	246.1 ± 23.57	259.3 ± 23.72	217.6 ± 19.29	222.6 ± 22.67	<0.001	0.206	0.571	
Lean body mass, g	40	175.6 ± 15.62	209.3 ± 18.17	171.3 ± 14.12	183.8 ± 20.07	0.010	<0.001	0.057	
%Body fat	40	28.6 ± 3.82	19.02 ± 6.26	21.12 ± 4.04	17.37 ± 5.19	0.006	<0.001	0.071	
Heart, g	40	0.73 ± 0.066	0.87 ± 0.076	0.77 ± 0.073	0.99 ± 0.070	0.003	<0.001	0.039	<0.001
Lungs, g	39	1.08 ± 0.139	1.03 ± 0.152	1.00 ± 0.155	1.13 ± 0.142	0.804	0.361	0.038	<0.001
Liver, g	40	6.93 ± 0.762	7.77 ± 0.844	6.36 ± 0.813	7.93 ± 0.781	0.496	<0.001	0.131	<0.001
Right kidney, g	39	0.81 ± 0.076	0.89 ± 0.085	0.89 ± 0.085	0.94 ± 0.079	0.029	0.015	0.615	<0.001
Spleen, g	38	0.44 ± 0.054	0.40 ± 0.063	0.48 ± 0.060	0.43 ± 0.057	0.116	0.036	0.849	<0.001
Stomach, g	39	1.11 ± 0.108	1.24 ± 0.120	1.09 ± 0.111	1.13 ± 0.108	0.090	0.017	0.206	<0.001
Small intestine									
Length, cm	40	110.2 ± 6.42	108.1 ± 7.12	105.0 ± 6.86	109.4 ± 6.58	0.436	0.564	0.110	0.011
Mass, g	40	5.58 ± 0.898	7.00 ± 0.996	5.43 ± 0.958	7.40 ± 0.920	0.722	<0.001	0.329	0.002
Large intestine									
Length, cm	40	23.8 ± 1.96	25.88 ± 2.15	21.9 ± 2.18	22.4 ± 2.02	0.001	0.048	0.226	0.514
Mass, g	40	2.38 ± 0.310	2.83 ± 0.367	2.31 ± 0.332	2.78 ± 0.316	0.624	<0.001	0.904	<0.001

Values are means ± SD. Significance of main effects, selected line × wheel access interaction, and body mass covariate was tested by ANOVA or ANCOVA. Effects that are significant at $P < 0.05$ are shown in boldface.

environment interaction. Mass-adjusted heart and lung mass showed significant selected line \times wheel access interactions (Table 3). In both cases, the increase in relative mass in groups with wheel access was greater in the HCR line. Presentation of the relative change in organ masses, expressed as percent difference, highlights the relatively small degree of evolutionary plasticity in organ mass compared with self-induced phenotypic plasticity (Table 4).

DISCUSSION

Although the behavioral motivation for voluntary wheel running in rodents is unclear (45), its role as a physiological stimulus has been well documented. Indeed, voluntary wheel-running activity is considered a useful model of self-training, because, given access to running wheels, numerous species of rodents will run many kilometers per day through a series of short high-intensity bouts (4, 6, 44, 49). Chronic (≥ 8 wk) exposure to running wheels results in hypertrophy and modification of metabolic capacity of cardiac and skeletal muscle in rats (43, 44, 56) and mice (1, 15). Additionally, we and others have found that voluntary wheel-running activity elicits a variety of physiological modifications in rodents, including decreases in body mass and body fat (50, 51), as well as increases in $\dot{V}O_{2\max}$ (28, 49, 56), muscle metabolic capacity (18, 57), and modulation of metabolically relevant endocrine functions, such as those governed by the hypothalamus-pituitary-adrenal axis (53). Here, we demonstrate significant modification of body composition, including the relative masses of internal organs involved with nutrient extraction and utilization, in response to chronic daily exercise.

Indeed, rats selectively bred for high and low treadmill-running capacity provide an example of "self-induced adaptive plasticity." We show that, when given access to running wheels, HCR rats display multiple morphological changes to support greater aerobic output (15, 17, 48), including "lean"

body composition and larger heart and kidney masses. When housed under standard cage conditions, the HCR rats exhibit higher physical activity levels, which may lead to some level of self-training that results in increased energy expenditure, lower body fat, and other lean characteristics consistent with a high-endurance phenotype (38, 53). When housed with access to running wheels, expression of behavioral activities for diet and exercise increased and, thus, the physiological characteristics (heart mass, lung mass, and fuel consumption/utilization) that would benefit a high-endurance phenotype were sharpened, in HCR rats. Such a positive-feedback loop between high-activity and high-endurance phenotypes should serve to reinforce and strengthen the correlation between the two inter-related traits. Whether chronic wheel exposure would increase the difference between the HCR and LCR rats in treadmill endurance capacity has yet to be determined.

Wheel running. We found that HCR rats ran significantly more minutes per day than LCR animals (Table 1) and that HCR rats tended to run at somewhat, but not statistically significantly, higher speeds. This resulted in a trend toward the HCR rats running greater total distances during the 2 wk of the feeding trial. This is not surprising, as these results are qualitatively similar to the wheel-running analysis for the entire duration of the experiment (53), where we found significant differences between LCR and HCR rats for time spent running and total distance run. Here, smaller sample sizes ($n = 17$ with wheel access in current study and $n = 29$ with wheel access in larger study) and a restricted 2-wk window within an 8-wk wheel-access period appear to have reduced the statistical power to detect differences in overall activity between the selected lines (see Ref. 53 for details of the methods and the 8-wk repeated-measures design).

Food consumption. Our results suggest that the cost of wheel running, as indicated by a 34% increase in body mass-adjusted food consumption across both lines, represents a substantial portion of the energy budget of laboratory rats, contrary to models that suggest otherwise (24, 51). In the HCR line, rats with access to running wheels consumed 46% more food per day than sedentary individuals. In the LCR line, rats with wheel access consumed 22% more food per day than those without wheel access. This difference was significantly less than that seen in the HCR lines, as indicated by the significant selected line \times wheel access interaction (Table 2). These values are qualitatively similar to those reported between lines of mice selected for increased wheel-running activity for 13 generations (24.5% increase) and their control lines (19.5% increase) (51). Although the increases in food consumption associated with wheel access are substantial, they are modest compared with those associated with altitude ($\sim 50\%$) (12), cold exposure ($>100\%$) (22, 26), lactational performance ($>100\%$) (10), or combinations of energetic demand ($>100\%$) (11) that have been used in previous studies of visceral organ phenotypic flexibility.

The presence of a significant selected line \times wheel access interaction implies a genotype \times environment interaction. Interestingly, the increase in food consumption appears to be greater in these endurance capacity rat lines, even though the relative difference in wheel-running activity was smaller between the HCR and LCR lines ($\sim 60\%$) than between the high-activity and control mouse lines at *generation 13*, when food consumption was measured ($>200\%$), and this result may

Table 4. Data and statistical results from Table 3 expressed as percent differences to illustrate relative magnitude of evolutionary and self-induced phenotypic plasticity

	Percent Difference		
	Sedentary	Sedentary vs. wheel access	
	LCR vs. HCR	LCR	HCR
Heart mass	5.5*	19.2 \dagger ‡	28.6 \dagger ‡
Lung mass	-7.4	-4.6‡	13.0‡
Liver mass	-8.2	12.1 \dagger	24.7 \dagger
Right kidney mass	9.9*	9.9 \dagger	5.6 \dagger
Spleen mass	9.1	-9.1 \dagger	-10.4 \dagger
Stomach mass	-1.8	11.7 \dagger	3.7 \dagger
Small intestine			
Length	-4.7*	-1.9	4.2
Mass	-2.7	25.4 \dagger	36.3 \dagger
Large intestine			
Length	-8.0	8.8 \dagger	2.3 \dagger
Mass	-2.9	18.9 \dagger	20.4 \dagger

To assess direction and magnitude of evolutionary plasticity, relative size of HCR organ masses compared with LCR organ masses was tabulated. To assess direction and magnitude of self-induced phenotypic plasticity, relative size of organs of animals with wheel access were compared with their sedentary counterparts within LCR and HCR lines. *Selected line effect ($P < 0.05$). \dagger Wheel access effect ($P < 0.05$). \ddagger Selected line \times wheel access effect ($P < 0.05$).

be explained by differences in the wheel-running phenotype. In the high-running-activity mouse lines, the increase in total activity was accomplished primarily via increased average running velocity (24). Whereas in the HCR line the observed increase in total activity was accomplished primarily via increased running time (53), which is expected to result in a substantial increase in energy expenditure, because a significant portion of the cost of locomotion is independent of running speed (see Ref. 24 and Fig. 6 in Ref. 24). Rats from the HCR line not only exhibited lower efficiency of digestion than rats from the LCR line, but they also exhibited decreased digestion efficiency when given access to running wheels, while efficiency increased for rats from the LCR line with wheel access. Apparently, HCR rats compensated for increased energy demand behaviorally by increasing food consumption to a greater degree than LCR rats, which, barring increases in morphophysiological traits related to energy acquisition, may place constraints on maximum rates of energy assimilation (24). We found that LCR and HCR rats with wheel access and greater energy demands wasted a greater proportion of their food (orts) (Table 2). However, a previous study in mice found the opposite pattern: food wasting decreased when animals were faced with greater energy challenges of cold exposure and lactation (23).

Previous studies (24, 51) report that food consumption, when adjusted for body mass, was positively associated with individual variation in running activity, including variation in revolutions per day and amount of time spent running per day; not surprisingly, animals that run more expend more energy and require more food. In the present study, access to running wheels clearly increases food consumption. However, the small sample sizes in this study prohibited a formal statistical test of the effects of individual variation in wheel running (i.e., daily activity as a covariate) on food consumption and body composition. It would be interesting to determine whether, as was found for lines of mice selected for high levels of wheel-running activity (24), these rats, which differ intrinsically for exercise capacity, also experience higher energetic costs associated with incremental increases in time spent running compared with costs associated with incremental increases in distance alone.

Selection for high-endurance running capacity resulted in greater mass-adjusted daily food consumption, independent of whether the rats had access to running wheels. The effect of selected line on food consumption was modest in the sedentary animals. HCR rats consumed $\sim 5.5\%$ more food per day, after correction for body mass (Table 2). These differences between LCR and HCR for mass-corrected daily food consumption under sedentary conditions persist and even appear to continue to diverge with sustained bidirectional selection (37, 38).

Our experiment clearly demonstrates a much greater increase in food consumption by the HCR rats with access to wheels than by their sedentary counterparts and by LCR rats. These results suggest that correlated responses in food consumption can be explained by correlated changes in behavior that increase energy demand, correlated changes in underlying physiological traits associated with metabolism, or a combination of both. For example, the modest food intake difference between lines without access to running wheels might be partially explained if HCR rats are more active than controls, even when they are not housed with activity wheels. Recent

data indicate a positive correlation between endurance capacity and home cage activity. Novak et al. (38) found a 25% increase in activity, counted as beam breaks over a 24-h period, and suggested that this heightened activity could explain the differences in food consumption. Similarly, in the high lines of the mouse wheel-running selection experiment, home cage activity was increased, compared with control lines, even when no running wheels were provided (31, 41). Taken together, these results strongly suggest that increases in baseline activity almost certainly explain at least part of the increase in daily food consumption. A similar pattern of increased home cage activity has also been observed in the high-basal metabolic rate (BMR) lines described above (8).

Alternatively, these differences in food consumption might at least partially result from correlated responses in underlying physiological traits related to metabolism. The HCR rats exhibited increased $\dot{V}O_{2\max}$ compared with LCR rats (9). If the HCR rats also evolved higher resting metabolic rates, this phenomenon could partly explain some of the differences in food intake in the absence of differences in home cage activity. Given the increased percentage of lean mass and increased mass of metabolically active visceral organs (see below), it seems reasonable to suspect that HCR rats have a higher BMR.

Body mass and body composition. Consistent with a previous report (20), we found that body mass was smaller in HCR than LCR rats at the beginning and end of 8 wk of wheel access. Although wheel access did not significantly alter total body mass, it did have significant and opposite effects on percent body fat and lean body mass. Regardless of selection history, access to running wheels significantly reduced accumulation of total body fat while increasing lean mass (Table 3). Independent of the wheel-running regimen, HCR rats were leaner, exhibiting lower fat-free body mass and a lower percent body fat, reinforcing previous reports, based on dissections of visceral fat pads, that HCR rats were leaner (37, 55). Similarly, body mass was smaller and percent fat was lower in mice from the high-wheel activity lines than control lines (39). In BMR-selected mouse lines, the high-wheel activity lines did not differ from the low-wheel activity lines in body mass, but the proportion of their mass that comprised lean tissue, as estimated by total body electrical conductivity, was significantly higher (27).

Nehrenberg et al. (36) reported that the relationship between wheel-running exercise and change in body fat in rodents was highly dependent on genetic selection history. Mouse strains selectively bred for wheel running or body composition showed a significant relationship, whereas a common inbred and outbred strain did not. Interestingly, we also found, among a large population (47 females and 50 males) of unselected N:NIH rats, a significant dependence of intrinsic aerobic running capacity on visceral fat pad (epididymal and perimetrial) compared with other organ structures (see supplemental Table S1 and Fig. S2). Every 2% increase in visceral adiposity resulted in ~ 80 m less maximal running distance on treadmill-running test to exhaustion [females: $y = -390x + 918$ ($r = -0.30$, $P = 0.04$); males: $y = -409x + 968$ ($r = -0.40$, $P = 0.004$)].

A growing number of intraspecific studies in birds and mammals have found significant correlations between whole organism aerobic traits such as BMR, maximal metabolic rate, and subordinate visceral organ masses after statistical correc-

tion for the effects of body mass (see Table 7 in Ref. 3 for a review). This result is not unexpected, given the role of these organs in acquiring and delivering energy. In addition to overall body fat and lean tissue composition, our current study also shows that selection for increased endurance capacity is accompanied by increases in mass-adjusted heart and kidney mass, organs that are associated with increased metabolic capacity. No other significant changes in mass-adjusted organ masses linked to an increase in metabolic machinery were observed in these lines (Table 3). These results are consistent with the finding that a variety of central and peripheral determinants of endurance capacity have increased concomitantly, but at different rates, across generations (9, 21). Swallow et al. (52) observed an increase in relative heart mass in lines of mice selected for increased wheel-running activity, but, unfortunately, they did not investigate organs of the GI tract. In contrast, Ksiazek et al. (26) found significant increases in body mass-adjusted small intestine, kidney, and heart mass in lines selected for increased BMR. According to the aerobic capacity model of the evolution of endothermy, it is hypothesized that the high BMR underlying endothermy evolved as a correlated response to the selection on $\dot{V}O_{2\max}$, because BMR and $\dot{V}O_{2\max}$ are genetically correlated (16). Therefore, if this theoretical model is correct, as selection for increased treadmill endurance in the HCR rats continues, correlated changes in other visceral organ masses may surface, assuming further increases in $\dot{V}O_{2\max}$ and the associated changes in daily energy demand (21).

Inspection of Table 4 illustrates that although genetic flexibility with regard to organ mass was limited, the magnitude of self-induced phenotypic modification of traits associated with energy acquisition and delivery in response to wheel access was much greater. After 8 wk of access to running wheels, we found widespread hypertrophy of the energy acquisition organs of the GI tract and organs of energy delivery in both lines, including increases in body mass-adjusted heart, kidney, and liver mass. Our results are important, because they demonstrate that relatively modest and self-induced increases in long-term energy demand and delivery result in the upregulation (see also Ref. 35) of phenotypic flexibility of visceral organs that had previously been demonstrated only in the face of considerably greater energy challenges, including cold (22, 25, 26), altitude (12, 13), and lactational stress (10). Furthermore, we found genotype \times environment interactions in HCR rats associated with heart and lung mass, indicating greater training responses in organs that sustain a repetitive intrinsic contraction rhythm for energy transfer. The degree of GI tract hypertrophy was not greater in the HCR than LCR lines, which may explain the genotype \times environment interactions related to food consumption and efficiency of digestion. Perhaps GI tract hypertrophy is less tightly linked to endurance capacity and long-term energy expenditure, because HCR rats were able to behaviorally compensate by consuming more calories. Lastly, the fact that digestibility decreased in the HCR line suggests that hypertrophy did not completely track with increased food consumption. Overall, the data presented here provide a starting point for a more in-depth analysis of the cellular mechanisms behind the observed responses, in particular, what changes in gene/protein expression networks may be driving these differences between HCR and LCR lines in the sedentary and physically active states.

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DISCLAIMER

The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of NSF, NCRR, or NIH.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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