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ORIGINAL ARTICLE

Western diet increases wheel running in mice selectively bred for high voluntary wheel running

TH Meek[1], JC Eisenmann[2] and T Garland Jr[1]

[1]*Department of Biology, University of California, Riverside, Riverside, CA, USA and* [2]*Department of Kinesiology, Michigan State University, East Lansing, MI, USA*

Objective: Mice from a long-term selective breeding experiment for high voluntary wheel running offer a unique model to examine the contributions of genetic and environmental factors in determining the aspects of behavior and metabolism relevant to body-weight regulation and obesity. Starting with generation 16 and continuing through to generation 52, mice from the four replicate high runner (HR) lines have run 2.5–3-fold more revolutions per day as compared with four non-selected control (C) lines, but the nature of this apparent selection limit is not understood. We hypothesized that it might involve the availability of dietary lipids.
Methods: Wheel running, food consumption (Teklad Rodent Diet (W) 8604, 14% kJ from fat; or Harlan Teklad TD.88137 Western Diet (WD), 42% kJ from fat) and body mass were measured over 1–2-week intervals in 100 males for 2 months starting 3 days after weaning.
Results: WD was obesogenic for both HR and C, significantly increasing both body mass and retroperitoneal fat pad mass, the latter even when controlling statistically for wheel-running distance and caloric intake. The HR mice had significantly less fat than C mice, explainable statistically by their greater running distance. On adjusting for body mass, HR mice showed higher caloric intake than C mice, also explainable by their higher running. Accounting for body mass and running, WD initially caused increased caloric intake in both HR and C, but this effect was reversed during the last four weeks of the study. Western diet had little or no effect on wheel running in C mice, but increased revolutions per day by as much as 75% in HR mice, mainly through increased time spent running.
Conclusion: The remarkable stimulation of wheel running by WD in HR mice may involve fuel usage during prolonged endurance exercise and/or direct behavioral effects on motivation. Their unique behavioral responses to WD may render HR mice an important model for understanding the control of voluntary activity levels.
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Keywords: artificial selection; exercise; genetics; genotype-by-environment interaction; sensitivity; voluntary activity

Introduction

Understanding the biological basis of voluntary physical activity has been an important goal of both biomedicine[1] and evolutionary physiology.[2] In addition to biological effects, environmental factors can modulate both physiology and behavior, and effects can be nonadditive, that is, show genotype-by-environment interaction.[3] Diet is a highly variable environmental factor (although behavior and physiology affect dietary choices), and elucidating the role that dietary macronutrients have in providing energy during locomotion and other physiological processes should shed light on the current human obesity epidemic.[4,5] Furthermore, comparative, ecological and evolutionary physiologists are attempting to reveal how dietary physiology may interact with, and even constrain, behavioral ecology and evolution.[2,6,7]

In this study, we investigated genotype-by-environmental interactions and potential limitations that diet places on locomotor behavior through the use of an artificial selection experiment using laboratory house mice. Starting in 1993, mice were bred for high voluntary wheel-running behavior (a major component of total voluntary locomotor activity when wheels are available), and after 16 generations, individuals from the four replicate high runner (HR) lines ran 2.5–3.0-fold as many

Correspondence: Dr T Garland, Department of Biology, University of California, Riverside 92521, CA, USA.
E-mail: tgarland@ucr.edu
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revolutions per day as compared with those from four non-selected control (C) lines, a differential that has continued through more than 36 subsequent generations of selection.[8,9] Various changes in locomotor performance, behavior and neurobiology (especially related to motivation for wheel running) have been observed in the HR lines.[10–12] For instance, treadmill endurance capacity is elevated in HR mice,[13] as is maximal oxygen consumption.[14]

Despite the continued selection since generation 16, HR mice seem to be at a selection limit, the biological causes of which are not yet clear.[9,15] Several pharmacological studies have been performed, and some drugs increase wheel running in the C lines; however, no drug has increased running in the HR lines.[8,10]

One possible factor that could limit further increases in wheel running by the HR lines is the availability of necessary energy substrates. The HR mice run for $\sim 6\,h\,day^{-1}$, virtually all at night,[15] and in mammals, generally, this type of sustained aerobic exercise should be supported primarily by lipid oxidation.[6,16] At low exercise intensities, lipids, in the form of fatty acids, predominate as the cellular fuel source. As exercise intensity increases, cellular triglycerides, plasma glucose and cellular glycogen also provide essential energy to maintain exercise performance.[16] In untrained human beings, half the energy used to exercise at 65% of maximal oxygen consumption (VO_2max) comes from lipids in the form of both free fatty acids and muscle triglycerides.[16] In the context of enhancing endurance performance, ingesting carbohydrates during exercise is often emphasized, but diets rich in lipids can also lead to increased performance at low or moderate intensities in rodents[17–19] and in humans,[20,21] although not always.[22]

Recent evidence has shown that HR mice do not deplete liver or gastrocnemius muscle glycogen stores any more than controls during nightly wheel running, nor do they have elevated glycogen synthase activity.[9] Thus, glycogen depletion does not seem to limit further increases in running. Previous studies have also found that HR mice have less body fat than C mice, and even a lower % body fat than mice from a separate experiment that selectively bred for low body fat.[23] Reanalysis of mice from generation 14 (data from Houle-Leroy *et al*.[24]) indicates HR males have elevated carnitine palmitoyltransferase levels compared to C lines, suggesting a higher ability to oxidize fats.[9] Therefore, we hypothesized that insufficient dietary lipids may be one factor limiting further evolutionary increases in wheel running by the HR lines. As an initial test of this idea, we measured wheel running, food consumption (Teklad Rodent Diet (W) 8604, 14% kJ from fat, or Harlan Teklad TD.88137 Western Diet (WD), 42% kJ from fat) and body mass over 1–2-week intervals in 100 males for 2 months starting 3 days after weaning (21 days of age). We predicted that HR mice would exhibit elevated wheel running when provided WD.

Materials and methods

Experimental animals

Mice were sampled from the 52nd generation of an artificial selection experiment for high voluntary wheel running (for reviews, see Rhodes *et al*.;[10] Swallow *et al*.[12]). The original progenitors of the selection experiment were outbred, genetically variable Hsd:ICR house mice (*Mus domesticus*). After two generations of random mating, 10 pairs of mice were used to create each of eight closed lines (that is, no interbreeding between lines was allowed). Four lines are bred for high running on wheels (HR lines) and four are bred without regard to wheel running (C lines). In each generation, mice are housed individually in standard cages ($27 \times 17 \times 12.5$ cm) attached to Wahman-type activity wheels (1.12 m circumference, 35.7 cm diameter, 10-cm-wide running surface) when they reach 6–8 weeks of age. Wheels are interfaced to a computer and revolutions are recorded in 1-minute intervals, continuously for 6 days; the selection criterion is the number of revolutions run on days 5 and 6. Within each HR family, the highest-running male and female are chosen as breeders to produce the next generation. Within C families, a male and female are chosen without regard for running. Sibling matings are disallowed in all lines. Room temperature is maintained at ~72 Fahrenheit and photoperiod is 12:12 h, with lights on at 0700 Pacific Time. Water and food (Harlan Teklad Laboratory Rodent Diet [W]-8604) are available *ad libitum*. Pregnant dams are given a breeder diet (Harlan Teklad Mouse Breeder Diet [S-2335] 7004) through weaning.

For this study, 100 male mice from generation 52 were weaned at 21 days of age, and then singly housed with access to Harlan Teklad Laboratory Rodent Diet [W]-8604 until they reached 24 days of age.

Procedures

At an average of 24 ± 0.2 (s.e.) days of age (experimental day 1), mice were housed individually with access to wheels (as described above). We used young mice because this was part of a larger study aimed at examining the ontogeny of voluntary locomotor activity and biomarkers of the metabolic syndrome. Half the mice continued to receive the standard diet (s.d.; Harlan Teklad Rodent Diet [W] 8604, 14% kJ from fat) and the other half received WD(Harlan Teklad TD.88137 WD, 42% kJ from fat; Table 1).

Mice and food hoppers were weighed on experimental days 1, 8, 16, 30, 43 and 58. We measured (apparent) food consumption as the difference in hopper mass between two time points, after accounting for any obvious wastage.[25] As the diets differ in mass-specific energy content, we converted food consumption to caloric intake, using the values shown in Table 1.

Total wheel running (revolutions) was recorded in one-minute bins for approximately 23 h every day. From these records, we computed weekly or bi-weekly averages (to

Table 1 Composition of the diets

	Standard diet (8604)		*Western diet (88137)*	
	g kg^{-1}	*%kJ*	*g kg^{-1}*	*%kJ*
Total protein	245	33	173	15
Added casein	0		195[a]	
Total fat	45	14	212	42
Saturated	10		133	
Monounsaturated	11		59	
Polyunsaturated	21		9	
Total Carbohydrates	409	53	485	43
Added sucrose	0		341	
Energy content	13.0 KJ g^{-1}		19.0 KJ g^{-1}	

Diet composition of both standard and western diets. Percent kJ of total protein, fat and carbohydrates are provided for each diet and g kg^{-1} measures are shown with additional nutritional information of dietary components. [a]Added casein in g kg^{-1} is greater then the total protein content of the diet because casein itself is only ~87% protein. The remaining content composing casein comes from fat (~1%), ash (~1%), and moisture (~11%).

match periods over which food consumption was recorded) for wheel running (revolutions per day), the number of 1-min intervals with at least one revolution (min per day), and mean revolutions per min for the active intervals (r.p.m.).

After 8 weeks of the experimental protocol, mice were weighed and body length was measured as nose-to-rump length of live mice before killing by decapitation. The retroperitoneal fat pad was then dissected and weighed.

Statistical analyses

Analyses were performed using the Mixed Procedure in SAS 9.1.3 (SAS Institute, Cary, NC, USA) to apply analysis of covariance models with Type III tests of fixed effects. Linetype (HR or C) and mini-muscle status (see next paragraph) were treated as fixed effects; line was nested within line type as a random effect. Effects of linetype, diet and the linetype–diet interaction were tested relative to the variance among replicate lines, and degrees of freedom were always 1 and 6. Covariates depended on the trait analyzed and included age, body length or body mass, wheel freeness (an inverse measure of rotational resistance) and total wheel running. Dependent variables and/or covariates were transformed as necessary to improve the homogeneity of the spread of the covariates, linearity of relations and/or normality of residuals.

One unexpected feature of the selection experiment has been the increase in frequency in two of the four HR lines of a small-muscle phenotype, termed mini-muscle and characterized by an approximately 50% reduction in triceps surae and whole hind limb muscle mass (see study by Houle-Leroy *et al*.[24] and references therein). Pleiotropic effects of this Mendelian recessive allele are many and varied, and include alterations in muscle fiber-type composition (fewer type IIb fibers),[26] contractile properties,[27] a doubling of mass-specific aerobic capacity and hexokinase activity,[14,24] increased glycogen concentration in gastrocnemius,[9] increased myoglobin concentration in medial gastrocnemius,[14] and increased heart ventricle mass.[13] Mini-muscle individuals run faster on wheels, and run more total revolutions per day under some conditions.[9,27] Thus, we included mini-muscle status as an independent variable in all analyses that involved the HR lines.

As we found significant linetype–diet interactions for several traits, separate analyses of HR and C lines were also performed. In these analyses, line was treated as a fixed effect. Although differences among the replicate lines (or line–diet interactions) were found for several traits, they are not the focus of this report and so are not discussed here, and we simply report the effects of diet.

Results

Body mass and retroperitoneal fad pad mass

Analysis of body length indicated no statistical effect of linetype, diet, their interaction or mini-muscle status (all $P>0.43$). The HR mice tended to be lighter in body mass than C mice, especially during the later weeks of the experiment and regardless of diet (Figure 1), although the difference was not statistically significant at any measurement time (results not shown). The linetype–diet interaction was never close to significant (all $P>0.73$), and WD increased body mass (Figure 1), the effect reaching statistical significance ($P<0.05$) at weeks 2 and 8.

With log body length as a covariate, WD significantly increased retroperitoneal fad pad mass, and HR mice were leaner than C, with no linetype–diet interaction (Table 2). When wheel running was included in the model as an additional covariate, the effect of diet remained, but the difference in body fat between HR and C mice was eliminated (Table 2). Thus, the reduced fat pad mass of HR mice can be explained, at least in statistical terms, by the intermediate phenotype of increased wheel running. Finally, we added caloric intake (log transformed) as an additional covariate. In this analysis, the effects of wheel running and diet remained highly significant (Table 2). Thus, WD *per se* increased fat pad mass in all mice, even after adjusting for caloric intake. In all three models, mini-muscle individuals had significantly larger fat pads (Table 2), despite tending to have reduced total body mass (results not shown).

Caloric intake

As shown in Table 3, body mass was always a highly significant, positive predictor of caloric intake. With body mass as a covariate, food consumption (kJ day^{-1}) was influenced by both linetype and diet, but the effects changed over the course of the experimental period (Figure 2a; Table 3). During the first week, WD increased energy intake of all mice by approximately 23%. By week 2 and continuing

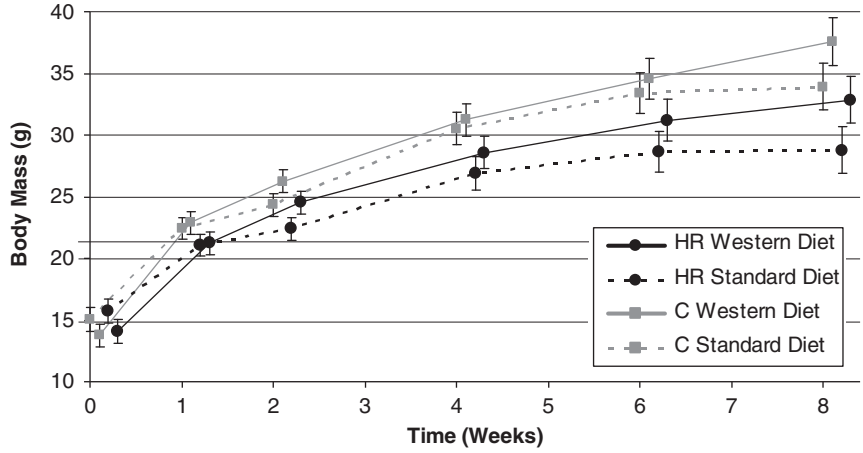

Figure 1 Least squares means and s.e. values for body mass of high runner (HR) and control (C) mice housed with access to wheels. At week 0, mice were 24-day old, given access to wheels and separated into diet groups. Points are staggered along the *x*-axis for visual clarity of s.e. values.

Table 2 *P* values from four alternate ANCOVA models of retroperitoneal fat pad mass (log transformed) with covariates

	P	P	P	P
HR vs C	**0.0283** [−]	**0.0263** [−]	0.6646 [−]	0.7542 [−]
Diet	**0.0007** [+]	**0.0007** [+]	**0.0005** [+]	**0.0001** [+]
Linetype × Diet	0.8222	0.7830	0.7388	0.8993
Mini-muscle	**0.0052** [+]	**0.0048** [+]	**0.0055** [+]	**0.0045** [+]
Log body length	**0.0078** [+]	**0.0133** [+]	**0.0425** [+]	0.2788 [+]
Wheel running			<**0.0001** [−]	<**0.0001** [−]
Log caloric intake		0.7249 [+]		**0.0100** [+]
AIC	−31.6	−31.5	−35.0	−40.6

Abbreviations: AIC, Akaike information criterion; ANCOVA, analysis of covariance; C, control; HR, high runner. Results are from two-way nested analysis of covariance models in SAS Procedure Mixed with categorical factors of linetype (HR vs. C), diet and mini-muscle status, and covariates of body length, wheel running and/or caloric intake. Replicate line was included as a random effect in all analyses. $N = 99$. Wheel running represents mean revolutions per day over the 2 weeks before killing. + indicates direction of effect, including HR > C, western diet > standard diet, mini-muscle > normal. Degrees of freedom are 1 and 6 for linetype (HR vs C), diet and their interaction. For tests of mini muscle, log body length, wheel running and caloric intake, degrees of freedom are 1 and 81, 80 or 79. All *P*-values are two-tailed. Significant values ($P < 0.05$) are in bold. AIC is Akaike Information Criterion, with smaller values indicating better-fitting overall model.

through the end of the experiment, the interaction between linetype and diet was significant, such that HR mice on WD were consuming more calories than all other groups, whereas C mice on WD reduced their caloric intake (Figure 2a). Mini-muscle individuals had significantly higher caloric intake during weeks 2–4 (Table 4).

When amount of wheel running (average revolutions per day) was included as a covariate, it always had a positive effect on caloric intake (as did body mass), and this effect was highly significant across weeks 3–8 (Table 4). In addition, amount of wheel running had substantial effects on the significance levels for some main effects during some measurement periods. In particular, during weeks 5–8, the inclusion of wheel running as a covariate eliminated the significantly higher caloric intake of HR mice relative to C mice, made the diet effect significantly negative and reduced the significance of the linetype–diet interaction (at least partly due to reduced effect sizes; Table 3; Figure 2b). Thus, after adjusting for both variation in body mass and amount of wheel running on an individual basis, caloric intake was reduced on WD for all mice during weeks 5–8.

For analyses of food consumption in $g\,day^{-1}$ (data not shown), values are consistent with previously reported data[25] and references therein. *P*-values for food consumption in $g\,day^{-1}$ were identical to those shown in Table 3 for each measurement period, with the exception of diet, which was highly significant at all weeks (all $P < 0.0001$), with or without wheel running as a covariate. In all cases, WD decreased food consumption measured as $g\,day^{-1}$.

Voluntary wheel running

The effect of WD on wheel running (revolutions per day) differed greatly between HR and C mice (Figure 3), and the linetype–diet interaction was statistically significant or nearly so across weeks 2–6 (Table 4). As expected from many previous studies of HR and C mice, in this sample, the daily wheel-running distance of HR mice was much higher than that for C mice when on standard diet, with the fold difference ranging from 2.2 to 4.0 (Figure 3b). Western diet increased this differential during all measurement periods, with the fold difference reaching a value as high as 5.7 during weeks 5–6 (Figure 3b).

As shown in Figure 3c, the effect of WD on wheel running was much greater in absolute terms (revolutions per day) in HR as compared with C lines. Separate analyses of the HR and C lines (data not shown) indicated that the revolutions per day of HR mice was significantly (two-tailed, $P < 0.05$)

Table 3 *P*-values from ANCOVA for caloric intake (kJ day^{-1}) with and without wheel running as an additional covariate

	Week 1 (n = 92)		*Week 2* (n = 99)		*Weeks 3 and 4* (n = 98)		*Weeks 5 and 6* (n = 98)		*Weeks 7 and 8* (n = 98)	
	P	P	P	P	P	P	P	P	P	P
HR vs C	0.4836 [+]	0.8699 [+]	0.2275 [+]	0.4531 [+]	0.0866 [+]	0.8238 [+]	**0.0195** [+]	0.7614 [−]	**0.0077** [+]	0.8990 [−]
Diet	<**0.0001** [+]	<**0.0001** [+]	**0.0101** [+]	**0.0228** [+]	0.8856 [+]	0.2834 [-]	0.4178 [−]	**0.0088** [−]	0.3905 [−]	**0.0216** [-]
Linetype × Diet	0.9527	0.7966	**0.0475**	0.0907	**0.0359**	0.0734	**0.0212**	0.0517	0.0672	0.2219
Mini-muscle	0.1784 [+]	0.1583 [+]	**0.0020** [+]	**0.0028** [+]	**0.0120** [+]	**0.0083** [+]	0.3361 [+]	**0.0111** [+]	0.6812 [+]	0.1225 [+]
Log body mass	<**0.0001** [+]	<**0.0001** [+]	<**0.0001** [+]	<**0.0001** [+]	<**0.0001** [+]	<**0.0001** [+]	<**0.0001** [+]	<**0.0001** [+]	<**0.0001** [+]	<**0.0001** [+]
Wheel running		0.2124 [+]		0.1980 [+]		<**0.0001** [+]		<**0.0001**[+]		<**0.0001** [+]

Abbreviations: ANCOVA, analysis of covariance; C, control; HR, high runner; WD, western diet. Results from two-way nested ANCOVA in SAS Procedure Mixed with categorical factors of linetype (HR vs C), diet and mini-muscle status, and covariates of body mass and wheel running. Replicate line was included as a random effect in all analyses. Food consumption analyzed as log (kJ day^{-1}). Log body mass was a covariate in all analyses, and age was a covariate in weeks 1, 2, 3 and 4 (results not shown). Models were analyzed both without and with wheel running (mean revolutions per day) as an additional covariate. + indicates direction HR > C, western diet > standard diet, mini-muscle > normal. Degrees of freedom are 1 and 6 for linetype (HR vs C), diet, and their interaction. For tests of mini muscle, log-transformed body mass, and wheel running, degrees of freedom are 1 and approximately 78. All *P*-values are two-tailed. Two outliers were removed from week-1 analysis and a single outlier was removed from all other weeks. Significant values ($P<0.05$) are in bold. For analyses of food consumption in g day^{-1} (data not shown), *P*-values were identical to those shown above for each measurement period, with the exception of diet, which was highly significant at all weeks (all $P<0.0001$), with or without wheel running as a covariate. In all cases WD, decreased food consumption measured as g day^{-1}.

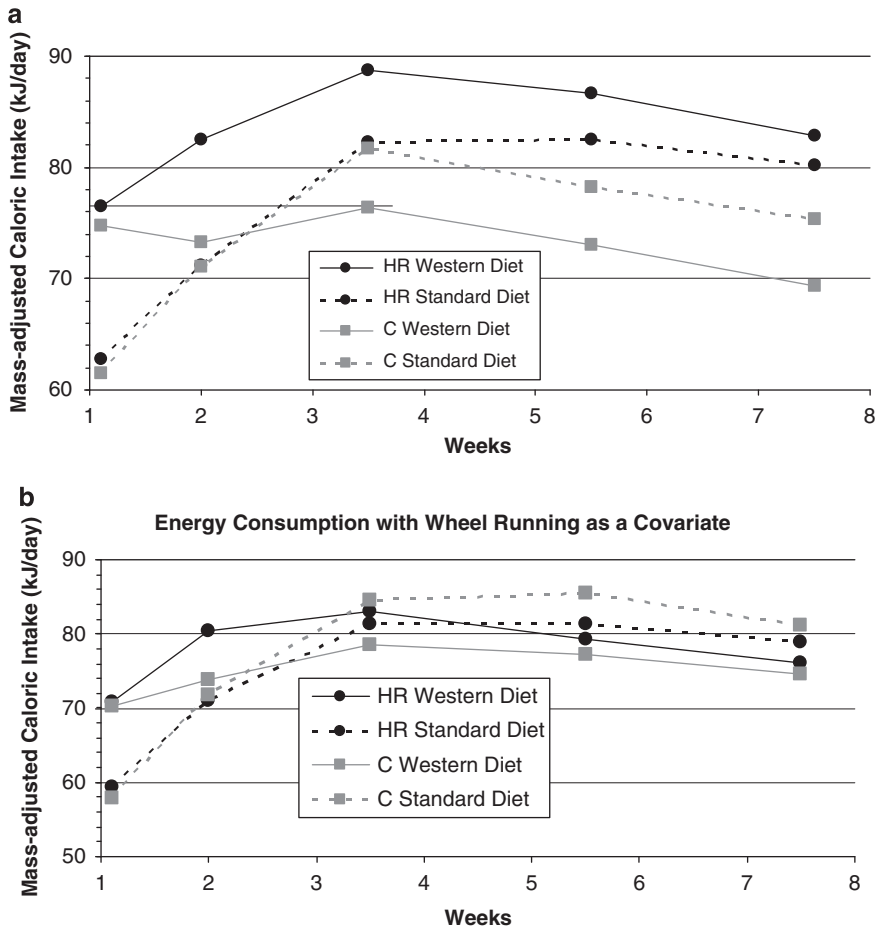

Figure 2 Mass-adjusted caloric intake (analysis of covariance (ANCOVA)) as measured in kJ/day (**a**). Values are least squares means, back transformed from log scale. Two outliers were removed from week 1 analysis and a single outlier was removed from all other weeks. In bottom panel (**b**), amount of wheel running was included as an additional covariate. The s.e. values are not presented as back-transformed values require separate upper and lower confidence limits, rather than s.e. values, which encumbers the visual presentation. See Table 3 for statistical results.

Table 4 *P*-values from ANCOVA for wheel running (revolutions per day)

	Week 1 (n = 96) P	*Week 2* (n = 100) P	*Weeks 3 and 4* (n = 100) P	*Weeks 5 and 6* (n = 99) P	*Weeks 7 and 8* (n = 99) P
HR vs C	**0.0010** [+]	**0.0038** [+]	**0.0012** [+]	**0.0012** [+]	**0.0057** [+]
Diet	**0.0266** [+]	**0.0039** [+]	**0.0343** [+]	0.1072 [+]	0.3195 [+]
Linetype × Diet	0.1281	**0.0106**	**0.0301**	0.0590	0.0709
Mini-muscle	0.8736 [−]	0.4441 [+]	0.9039 [+]	0.2090 [−]	0.5694 [−]

Abbreviations: ANCOVA, analysis of covariance; C, control; HR, high runner. Results from two-way nested ANCOVAs in SAS procedure mixed replicate line was included as a random effect in all analyses, and wheel freeness was used as a covariate (results not shown). Age was an additional covariate in analysis during weeks 1, 2, 3 and 4 (results not shown). + indicates direction HR > C, western diet > standard diet, mini-muscle > normal. Degrees of freedom are 1 and 6 for all tests, except for mini-muscle, where degrees of freedom were 1 and 77–81, depending on week. All *P*-values are two-tailed. Significant values ($P < 0.05$) are in bold.

increased by WD during all time periods, except weeks 7–8 ($P = 0.0878$). In contrast, WD had no statistical effect on running by C mice, except during the first week ($P = 0.0438$). On the basis of analyses of the HR lines, the stimulation of daily running distance was primarily a function of time spent running, which was increased by 66, 49, 34, 16 and 11%, respectively, across the five time periods ($P < 0.0001$, $P < 0.0001$, $P = 0.0009$, $P = 0.0877$ and $P = 0.2408$, respectively). Average running speeds also tended to be increased, with corresponding values of −1, 20, 17, 11 and 6% ($P = 0.8940$, $P = 0.0200$, $P = 0.0326$, $P = 0.1122$ and $P = 0.2998$).

To determine when the stimulation of running first occurred in HR mice, we analyzed individual days. Revolutions per day were increased by 58, 39, 58, 57, 70%, 60 and 56% on days 1–7, respectively ($P = 0.0321$, $P = 0.0832$, $P = 0.0160$, $P = 0.0086$, $P = 0.0014$, $P = 0.0073$ and $P = 0.0168$, respectively).

Discussion

Variation in physical activity levels may reflect underlying differences in both motivation for being active and physical abilities to engage in locomotor activity of particular intensities or durations. Here, we investigated the effect of a Western diet (WD; 42.0% of kJ from fat plus added sucrose) on voluntary wheel-running behavior by lines of mice (HR) that have been selectively bred for high wheel running. Since approximately generation 16, mice from the four replicate HR lines have run 2.5–3-fold more on a daily basis as compared with four replicate non-selected control (C) lines. In spite of continued selective breeding, running by the HR lines has not increased appreciably since generation 16.[9,12] In this context, our most dramatic finding is that WD caused a large increase in daily wheel running of HR mice, with little or no effect in C mice (Figure 3). This supports our hypothesis that dietary lipids may be constraining further evolutionary increases in wheel running. No other pharmacological or environmental agent has had this dramatic, positive effect on running by HR mice.[8,10] The reason for this remarkable difference in response to WD between HR and C mice is unclear, but may involve WD's role in providing necessary lipids to help sustain prolonged submaximal exercise and/or stimulating areas in the brain involved in motivation for, or reward received from, wheel running (see below).

To the best of our knowledge, no other studies have examined the effect of this particular 'Western diet' (Table 1) on any aspect of voluntary locomotor activity in mice. However, studies examining voluntary activity in animals fed high-fat diet show a variety of responses. In a study using mice bred for low (L) or high (F) % body fat, Simoncic *et al*.[28] reported that L% body fat mice did not change their total running distance on being fed high-fat diet. The F% body fat mice increased wheel running compared with F% body fat mice on regular chow, but running distances did not exceed values of L% body fat animals on either diet (Figure 3). Cheng *et al*.[29] reported that male Long-Evans rats fed high-fat diet for up to 6 weeks did not show any significant differences in voluntary wheel-running distance compared with rats fed standard diet, despite the fact that rats administered with the high-fat diet showed increased activities of carnitine acyltransferase (also called carnitine palmitoyltransferase) and β-hydroxy-acyl-CoA dehydrogenase in soleus muscle. Other studies have reported no change in home-cage activity levels when on high-fat diet or a high-fat + high-sucrose diet for male C57Bl/6J and A/J mice.[30] In a study on one HR and one C line (that is, a subset of the lines that we studied), Vaanholt *et al*.[31] found that high-fat diet did not statistically increase home-cage activity in males or females that were housed without wheel access. Bjursell *et al*.[32] used a diet very similar (high fat, high sucrose, 19.6 kJ g^{-1}) to that used in this study and observed a decrease in home-cage activity for inbred C57Bl/6J mice. Thus, the stimulation of wheel running in HR mice fed WD is a new observation.

Trained endurance athletes (human and rodent) are able to oxidize more free fatty acids for fuel during exercise than untrained counterparts.[29,33–36] As compared with C mice, HR mice exhibit several differences that are similar to those observed in trained athletes, including higher endurance[13]and aerobic capacity,[14] increased insulin-stimulated glucose uptake in isolated extensor digitorum longus muscle,[37] and higher carnitine palmitoyltransferase activity in mixed hind limb muscle.[9] Western diet may have affected

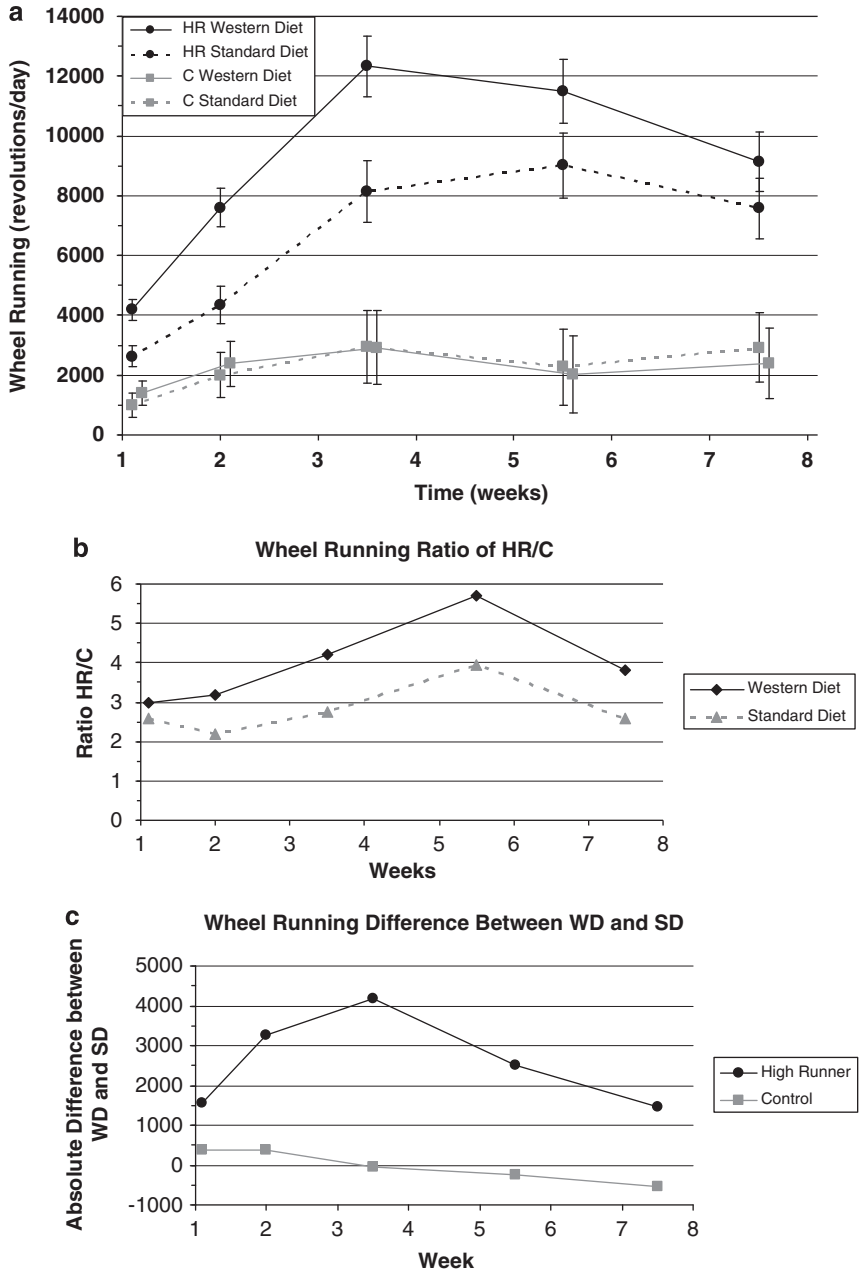

Figure 3 Least squares means and s.e. values for wheel running for both control (C; points staggered along the *x*-axis for clarity) and high runner (HR) mice on both diets. HR mice ran ~3-fold more than C mice when on standard diet, but this differential was greatly increased on Western diet. See Table 4 for statistical results.

the HR mice in this study so rapidly (within 1–3 days) because they are already primed to oxidize lipids. Moreover, as compared with C mice, HR mice show a much greater upregulation of GLUT-4 in gastrocnemius muscle within only 5 days of wheel access,[9] and it is possible that this occurs within 1–3 days. The foregoing characteristics suggest that HR mice, even in the untrained state, may be relying more on lipids during submaximal exercise, although previous respirometry studies have not reported significant differences from C in respiratory exchange ratio during rest or at maximal oxygen consumption during voluntary running.[15]

Even if HR mice are not primed to oxidize fats, if motivation is not limiting, then additional dietary lipids may lead to increases in running because diets high in fat have many physiological effects on cellular mechanisms that act to promote endurance performance capacity that would go

unused by C animals. A high-fat diet and elevated levels of plasma fatty acids lead to increases in the activity of citrate synthase, 3-hydroxyacyl-CoA dehydrogenase and carnitine palmitoyl-transferase[17,29,35,38] and induce increased biogenesis of mitochondria in skeletal muscle,[39,40] all of which would act to potentially increase fatty-acid metabolism. These effects can appear in only a matter of days, and in some cases reach their maximum levels within a week.[17,19] However, high-fat diets can also lead to higher levels of mitochondrial uncoupling protein (UCP3), which reduces the efficiency of energy production and, therefore, can impair exercise capacity.[41]

Although the precise limitation(s) on endurance exercise performance depend on the intensity and/or duration of the activity, the depletion of energy substrates is a commonly considered cause of fatigue. The ingestion, loading or use of particular dietary macronutrients is important in maintaining muscular activity and delaying fatigue during exercise. The alteration in the rate of use or balance of carbohydrates and lipids, often through training or diet, can lead to increased performance. For instance, Simi *et al*.[18] found high-fat diet acts in an additive manner with endurance training, leading to increased VO_{2max} and submaximal endurance in rats. High-fat diet can serve to increase the duration of moderate physical activity by sparing glycogen, the depletion of which is considered to be a cause of fatigue.[42] However, as HR mice do not seem to deplete liver or muscle glycogen to any greater extent than C mice when fed standard rodent chow,[9] this explanation seems unlikely for these wheel-running results. Nor does the WD's added sucrose seem likely to have any exercise-stimulating effects; when administered sucrose-based drinking solutions, both HR and C mice drank considerably more (compared with water), but showed no change in wheel running (EM Kolb and T Garland, unpublished results).

The physiological basis for the increased wheel running in HR lines appears to be different from mechanisms involved in the semipalmated sandpiper (*Calidris pusilla*), in which natural diets rich in *n*−3 polyunsaturated eicosapentaenoic acid (20:5) and *n*−3 docosahexaenoic acid (22:6) seem to increase the bird's oxidative capacity.[43] Both fatty acids lead to an increased cellular membrane fluidity and serve as ligands for peroxisome proliferators-activated receptors,[44] which are involved in regulation of lipid metabolism. Different types of fatty acids, however, are not identical in their effects on performance[45] or health.[4,46] There seem to be differences between polyunsaturated verses monounsaturated and saturated fats, and differences among types of polyunsaturated fats.[45,47] In our study, only 4% of the fat in the WD was polyunsaturated, with less that 0.5% coming from eicosapentaenoic acid and docosahexaenoic acid, whereas 64% was saturated fat, with 10.3, 29.4, 12.6% of total fatty acids coming from saturated fatty acid chains of 14, 16 and 18 carbons long, respectively. Therefore, if the running stimulation observed in HR mice occurs through direct or indirect effects on muscle physiology, then it seems likely that other fatty acid types besides polyunsaturated fats have key roles.

In addition to the many effects high-fat diet has on muscles, it has also been shown to stimulate certain brain areas (for example, hypothalamus) and signaling by certain neurotransmitters (for example, dopamine), putatively involved in reward[5,48–51] and possibly involved with the 'activitystat'.[52] Voluntary wheel running is also well known for its impact on the central nervous system, and is considered to be a classic self-rewarding behavior. Previous studies demonstrate that the reward circuitry, including dopaminergic and endocannabinoid pathways, have been altered in HR mice.[8,10,11] Therefore, it is possible that the motivation for wheel running and sensitivity of the 'activitystat' responds differently to a WD in HR as compared with C mice.

Recent research supports the presence of a neuro-hormonal system that maintains energetic balance and regulates total body weight.[53,54] Both mice and human beings seem to exhibit 'compensatory mechanisms' to deal with excess caloric intake, including decreased food intake, increased metabolic rate and increased non-exercise activity thermogenesis[28,31,53,55,56] (but see the review by Westerterp[57]). The increased wheel running by HR mice on WD (Figure 3) may represent an alternative compensatory mechanism.

Western diet (which has a high amount of saturated fats) led to increased adiposity (Table 2), despite decreased caloric intake during the latter part of the study (Table 3). The high palatability of fatty diets often leads to hyperphagia and thus weight gain, but not always.[58] Weight gain can occur in spite of decreased food consumption when on a high-fat diet, perhaps due to differences in the efficiency of storage or thermic effects of digestion.[19,32,58,59]

The WD used in this study has very high levels of saturated fats and added sucrose, both of which are considered unhealthy if ingested frequently because they can contribute to the metabolic syndrome and other chronic diseases. The impact this diet would have on health-related traits in these mice has not yet been explored, but the elevated running in HR mice may serve to ameliorate other negative effects of such a diet, much like wheel running reduced body fat in this study.

In conclusion, we would argue that an experimental evolution approach offers a powerful alternative to other models for studying the evolution of lipid metabolism (for review, see McClelland[6]). These results demonstrate that genetic selection history has a large impact on complex traits, such as locomotor behavior, susceptibility to weight gain and possibly central nervous system reward generated from exercise or eating. A better understanding of these traits is imperative for addressing obesity and the development of the metabolic syndrome.[60] Moreover, our results emphasize the importance of considering gene-by-environment interactions when studying the limits to sustained, relatively high-speed, voluntary locomotion. Further study will be required to determine the mechanism behind WD's unprecedented stimulatory effect on wheel running in the HR mice.

Conflict of interest

The authors declare no conflict of interest.

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