Physiology & Behavior 106 (2012) 252–258

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Effects of leptin treatment and Western diet on wheel running in selectively bred high runner mice

Thomas H. Meek *,[1], Elizabeth M. Dlugosz, Kim T. Vu, Theodore Garland Jr.

Department of Biology, University of California, Riverside, Riverside, CA 92521, USA

ARTICLE INFO

Article history:
Received 15 November 2011
Received in revised form 19 January 2012
Accepted 8 February 2012
Available online 16 February 2012

Keywords:
Artificial selection
Behavior
Genetics
High-fat diet
Locomotion
Voluntary exercise

ABSTRACT

The role of leptin in regulating physical activity is varied. The behavioral effects of leptin signaling depend on the type of activity and the animal's physiological state. We used mice from lines selectively bred for high voluntary wheel running to further study how leptin regulates volitional exercise. Mice from four replicate high runner (HR) lines typically run ~3-fold more revolutions per day than those from four non-selected control (C) lines. HR mice have altered dopamine function and differences from C in brain regions known to be important in leptin-mediated behavior. Furthermore, male HR mice have been found to dramatically increase running when administered Western diet, an effect possibly mediated through leptin signaling. Male mice from generation 61 (representing three HR lines and one C line) were allowed wheel access at 24 days of age and given either Western diet (high in fat and with added sucrose) or standard chow. After four weeks, Western diet significantly increased circulating leptin, insulin, C-peptide, gastric inhibitory polypeptide, and inflammatory hormone resistin concentrations in HR mice (C mice not measured). Western diet increased running in HR mice, but did not significantly affect running in C mice. During the fifth week, all mice received two days of intra-peritoneal sham injections (physiological saline) followed by three days of murine recombinant leptin injections, and then another six days of sham injections. Leptin treatment significantly decreased caloric intake (adjusted for body mass) and body mass in all groups. Wheel running significantly increased with leptin injections in HR mice (fed Western or standard diet), but was unaffected in C mice. Whether Western diet and leptin treatment stimulate wheel running in HR mice through the same physiological pathways awaits future study. These results have implications for understanding the neural and endocrine systems that control locomotor activity, food consumption, and body weight, and how they may vary with genetic background.

1. Introduction

Behaviors such as eating and engaging in volitional activity are regulated by a myriad of physiological and neurobiological interactions [1]. Eating and voluntary exercise also interact through their effects on homeostasis and by various direct mechanisms. In rodents, wheel access has been shown many times to increase food consumption, e.g. [2–5] although the effect does not always occur [6,7]. Severe underfeeding or food deprivation can result in substantial increases in locomotor activity, postulated to represent an increase in motivation for foraging behavior in mice and rats [8,9].

Different diets can have diverse effects on locomotor activity in rodents. However, determining to what extent variable effects are caused by different macronutrient compositions, the amount of energy ingested, differences among strains of rodents, changes in body mass or composition, or the type of locomotor activity measured has been challenging [3,10–13].

The role of leptin as an endocrine signaling molecule in both the periphery and central nervous system has become well-appreciated [14–16]. Many studies have focused on leptin's role in food consumption, thermoregulation, metabolic rate, and corresponding changes in body mass. Fewer studies have examined leptin's effect on physical activity, particularly voluntary exercise [17,18]. Both spontaneous physical activity (also known as non-exercise activity thermogenesis [NEAT]) and voluntary exercise can have large impacts on energy expenditure and consequently energy balance and body fat [19–21]. As both of these forms of activity can function as electively modifiable components of total energy expenditure, they may serve as major options for the treatment of obesity and many metabolic diseases.

To shed light on the role leptin may play in regulating levels of voluntary exercise, we studied mice from lines that have been selectively bred for high voluntary wheel running. Mice from the four replicate high runner (HR) lines typically run ~3-fold more revolutions per day than those from four non-selected control lines, and have evolved lower body size and lower body fat [22,23]. Circulating leptin

* Corresponding author. Tel.: +1 206 897 5286.
E-mail address: thmeek@uw.edu (T.H. Meek).
[1] *Present address*: 815 Mercer St., Department of Medicine, University of Washington Seattle, Seattle, WA 98109, USA.

0031-9384/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2012.02.012

concentrations are also lower in some studies of HR mice [24] (see also [25,26]) and, interestingly, leptin is lower than predicted based on fat mass, at least in young adult females [24]. As a corollary, HR mice also have elevated food consumption [5], but still remain leaner than controls [27]. It is unknown whether depressed leptin concentrations play a part in the motivation for increased wheel running, are a result of high activity, or are a correlated response to selection that is unrelated to activity levels per se.

The heightened wheel running of HR mice involves neurochemical changes affecting motivation and reward. Dopamine transporter blockers (Ritalin, cocaine, GBR 12909), which increase function of dopamine, reverse hyperactivity on wheels in HR mice, with an increase or no change in C lines. This suggests reduced functionality of the dopamine receptors in HR mice. Specifically, HR mice are more sensitive to blocking D1-like receptors rather than blocking D2-like receptors [28–30]. When wheel access is denied, the caudate–putamen complex, prefrontal cortex, nucleus accumbens, and lateral hypothalamus have differential activity in HR as compared with control mice (measured immunohistochemically using c-Fos) [31]. Not only are these brain regions involved in voluntary locomotion and/or motivation, but the latter two, along with the mesolimbic dopaminergic system, are known to be important in leptin-mediated behavior [14,32].

None of a number of previously-tested pharmacological agents increased running in HR mice [30,33], but administration of a Western diet increased daily wheel running in HR mice up to 75%, with no change in control mice [27]. In that study, both control and HR mice gained substantial fat mass when fed Western diet, and presumably circulating leptin concentrations rose as well. Given that HR mice have changes in brain regions involved in leptin signaling, differences in baseline circulating leptin concentrations, and respond uniquely to Western diet, we investigated leptin's role in modulating wheel running, and determined if leptin's effects were consistent between different diets.

2. Materials and methods

2.1. Experimental animals

Mice from generation 61 of an ongoing selection experiment for high voluntary wheel running were used. The original progenitors of the colony were outbred, genetically variable Harlan Sprague Dawley mice: Institute for Cancer Research strain (Indianapolis, Indiana, USA). Eight closed lines were formed, four selected for high voluntary wheel running (based on days 5 and 6 of a 6-day test) and four bred without regard to running [34].

58 male mice from generation 61 were weaned at 21 days of age and housed with access to Harlan Teklad Laboratory Rodent Diet [W]-8604 until they reached 24 days of age. One control line and three of the four selected HR lines were represented in this study as our focus was on elucidating the HR phenotype. The excluded HR line (lab designation #6) was polymorphic for the mini-muscle phenotype (see below). Room temperature was maintained at ~73 °F and photoperiod was 12:12, with lights on at 0700 Pacific Time.

2.2. Experimental groups

At 24 days of age, all mice were singly housed with Wahman-type wheels (1.12 m circumference, 35.7 cm diameter, 10 cm-wide running surface) attached to standard cages (27×17×12.5 cm) (Fig. 1). Wheel running was recorded for 23 h each day with the final hour (1200–1300) used to reset the computers, check for any wheel malfunctions, and check the health of the animals. Half the mice received standard diet (SD) (Harlan Teklad Rodent Diet [W] 8604, 14% kJ from fat) and the other half received Western diet (WD) with similar concentrations of vitamin D (Harlan Teklad TD.88137 Western Diet, 42% kJ from fat with added sucrose; see [27] for details of diet composition). Every six days mice were weighed, body length (tip of snout to base of tail) was taken, while the mouse was held behind the neck, and apparent food consumption measured. Food consumption was determined as the difference in hopper mass between two time points, after accounting for any obvious wastage. Our standard chow food consumption values are in agreement with a previous study using these lines that, in the absence of bedding, sorted, dried, and weighed all uneaten food to account for wastage [35]. Because the diets differ in mass-specific energy content, we converted food consumption from grams to caloric intake, using total kJ of metabolizable energy of 12.98 and 19.01 per gram of wet mass for SD and WD, respectively [27].

In some cases, a pair-fed group would provide clearer evidence as to the effects of caloric intake on the phenotype of interest. However, with regard to wheel running, we chose not to have a pair-fed group because limiting food intake in rodents can have profound effects to increase wheel running a response that is believed to represent foraging behavior [8,9]. If so, then the effect would be distinctly different from that of Western diet, and could have a more confounding than clarifying effect.

2.3. Blood sample

A 130 μl blood sample was taken after mice had been in experimental groups for two weeks (42 days of age). Blood was acquired through the orbital sinus under isoflurane anesthesia. Blood was collected in non-heparinized microcapillary tubes. 1.1 μl of dipeptidyl peptidase IV inhibitor (EC 3.4.14.5, Millipore MO, USA), 2.5 μl of 0.05 M phenylmethanesulfonylfluoride dissolved in methanol, and 10 μl of Roche mini Complete serine protease inhibitor cocktail (Roche Diagnostics Mannheim, Germany) were added to whole blood and mixed thoroughly. Serum was collected after blood was centrifuged (Sorvall Legend Micro 17R) at 13,000 rpm for 10 min at 4 °C.

Hormones were assayed using a Milliplex Mouse Metabolic Magnetic Bead Panel MMHMAG-44K-14 (Millipore MO, USA) in a Luminex 200. Standards were plotted and concentrations determined using Milliplex Analyst software version 3.5.5. Due to the limited number of wells, and to ensure adequate sample sizes in HR lines, only the three HR lines had blood assayed (blood samples from control mice were not assayed).

2.4. Injections

Starting at 24 days of age, mice were given 3 weeks of uninterrupted wheel access to allow daily wheel running to plateau before injections began. Recombinant mouse leptin (R&D Systems, Inc.) was prepared by dissolving in physiological saline immediately prior to use. Mice were given 2 μg/g body mass 2 h before lights off via intraperitoneal (i.p.) injections. Each mouse received two consecutive days of sham (physiological saline) followed by three consecutive days of leptin treatment and six more days of sham injections (Fig. 1). The mass used to adjust injection volume was measured during the initial two days of sham injection. Accordingly, even though leptin treatment changed body mass, injection volumes of leptin did not change over the course of the experiment. Injection volumes ranged from 0.12 ml to 0.17 ml.

2.5. Dissections

Mice were dissected one day after the final sham injection (26 days after the blood sample). Body mass, body length, and food consumption were recorded. The mouse was skinned and its pelt weighed. The ventricles, liver, and triceps surae were then dissected and weighed. Different fat pad masses were also dissected and weighed, including the subscapular brown fat with the sub-scapular adipose tissue, epididymal fat, and retroperitoneal fat [36]. For analyses, “total fat” refers to the sum of all fat pad masses.

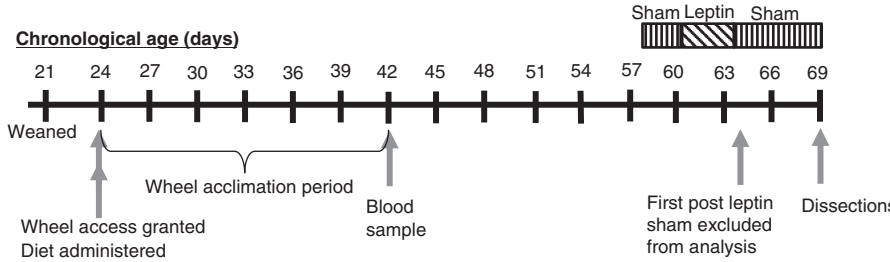

Fig. 1. Timeline of experimental design. Wheel access was granted to all mice from both linetypes (represented by three selectively bred HR lines and one non-selected control line) shortly after weaning, and half of each of the four lines received Western diet at this time. Every six days mice were weighed, body length was recorded, and food consumption measured. Wheel running was allowed to plateau before the blood sample was taken. All mice had blood drawn, but only HR animals had hormones assayed. After recovery, all mice received 2 days of sham (physiological saline), 3 days of leptin, then 6 days of sham injections intraperitoneally. Pretreatment running is reported for days 57–58. Experimental running is reported from days 59 to 69, excluding day 64 to allow for any possible carry-over effects of leptin treatment to diminish once final sham injections began.

2.6. Statistical analyses

Analyses were performed using the Mixed Procedure in SAS 9.1.3 (SAS Institute, Cary, NC, USA) to apply analysis of covariance (ANCOVA) models with Type III tests of fixed effects. Line and diet were main effects. Covariates depended on the trait analyzed and included body length, body mass, fat mass, wheel freeness, and/or total wheel running (revolutions). To compare values for food consumption, body mass, and wheel running, during the initial sham (2 days), leptin (3 days), and final sham injections (5 days), we analyzed the average values for each of these segments. A priori contrasts comparing sham with leptin values were used in SAS Procedure Mixed for a repeated-measures ANCOVA with covariates of body mass or wheel freeness and AR(1) rather than compound symmetry was used for covariate structure.

The three HR lines were averaged from the analysis with line as a main affect and, therefore, do not have S.E. bars. Baseline differences for body weight and caloric intake existed among the three HR lines, but their responses to experimental intervention were similar, so only the average value of all three lines is reported.

One of the three HR lines studied here (lab designation #3) is fixed for a Mendelian recessive allele that causes a small-muscle phenotype, with an approximately 50% reduction in triceps surae, as well as whole-hindlimb muscle mass [37,38]. Pleiotropic effects of this allele include alterations in muscle fiber type composition (especially reduced type IIB fibers) [39], contractile properties favoring stamina at the expense of speed [40], and a doubling of mass-specific aerobic capacity and hexokinase activity [38]. Mini-muscle animals showed similar increases in wheel running compared to normal muscle HR mice when given Western diet, so they are not distinguished in the results.

3. Results

3.1. Blood samples

As expected, plasma leptin concentrations were higher in mice fed Western diet (Table 1, Fig. 2). When total fat mass was included in the analysis as a covariate, it was highly significant and diet lost its significance. A similar pattern was observed for insulin. WD also increased C-peptide, which remained significantly elevated when fat mass was included in the model as a covariate. Resistin and gastric inhibitory polypeptide (GIP) were both higher in WD-fed mice, but monocyte chemotactic protein-1 (MCP-1) was not (Table 1, Fig. 2). The significant line effect for several hormones reveals baseline differences among the high runner lines. Despite these differences, their responses to Western diet are similar, as there is no significant diet × line interaction for any hormone (Table 1). When body fat is included as a covariate, the effect of line remains or becomes non-significant for all the hormones except GIP. Thus, the differences observed among HR lines may be attributable to differences in body fat.

3.2. Caloric intake

In the repeated-measures analysis (see Fig. 3) (with body mass as a covariate), leptin treatment decreased caloric intake (a priori contrast of initial sham vs. leptin injection period, $P<0.0001$). Once leptin injections stopped, caloric intake increased but did not quite reach baseline levels (a priori contrast of initial sham vs. final sham, $P=0.0104$). Both diet groups responded similarly to leptin injections (diet × time block interaction $P=0.1488$). The lines were different from each other ($P=0.0084$), with the control line eating less overall, and no statistical interactions with the line effect.

3.3. Body mass

Repeated-measures analysis showed that leptin treatment decreased body mass ($P<0.0001$, see Fig. 4). After leptin injections stopped, body mass increased to levels statistically indistinguishable from baseline values ($P=0.8786$). Western diet increased body mass ($P=0.0370$) of all lines (no line × diet interaction). Both diet groups responded to leptin injections in a similar way (no time × diet interaction). Lines differed significantly in body mass ($P=0.0146$), with all three HR lines weighing less than the one control line (results not shown; averaged values shown in Fig. 4). The response to leptin was similar among lines ($P=0.2316$ for the time × line interaction).

3.4. Wheel running

The two days before the start of sham injections, mice from HR lines were running ~3.3 times further per day relative to control mice. On this day, HR mice ran 20% more on Western diet ($P=0.0470$), which is comparable to the differential observed in our previous study when mice were this age [27]. Running of mice in the C line was not statistically affected by Western diet over these two days ($P=0.1117$).

In a repeated-measures analysis of the three HR lines, wheel running varied significantly across time blocks (Fig. 5, $P<0.0001$) and the effect of diet was not significant (and no interactions between diet and other factors). A priori contrasts showed that leptin increased running ($P=0.0035$ [+8%], $P<0.0001$ [+17%] compared to pre-leptin sham and post-leptin sham, respectively), and that mice ran less during the post-leptin sham than during the pre-leptin sham ($P=0.0388$ [−7%]). The time × line interaction was significant ($P=0.0308$), thus indicating somewhat different responses among the three HR lines (Fig. 5 panel B). However, inspection of the Least Squares Means from SAS showed that all three HR lines followed the same general pattern of increasing wheel running during leptin injections, followed by a decrease in running after injections stopped (Fig. 5 panel B). It is unclear why the different HR lines responded to hyperleptinemia to a varying extent. It could involve innate baseline differences in leptin levels, which can be accounted for by the slight differences in fat mass (Table 1). When the analysis is run with the

Table 1
P values from ANOVA and ANCOVA for plasma hormones in HR lines of mice.

	Leptin (n = 33)		Insulin (n = 38)		C-peptide (n = 38)		Resistin (n = 38)		GIP (n = 38)		MCP-1 (n = 21)	
	P	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*
Diet	**0.0031**[a]	0.5448[a]	**0.0002**[a]	0.0726[a]	<**0.0001**[a]	**0.0010**[a]	<**0.0001**[a]	<**0.0006**[a]	**0.0012**[a]	**0.0015**[a]	0.7025[a]	0.2492[aa]
Line	**0.0466**	0.2266	**0.0267**	0.3436	**0.0368**	0.2935	**0.0386**	0.0890	**0.0456**	**0.0352**	0.9291	0.4810
Diet × line	0.0620	0.9981	0.1797	0.8325	0.3588	0.1851	0.2106	0.2571	0.3309	0.1993	0.5885	0.7477
Fat mass		<**0.0001**[a]		**0.0002**[a]		**0.0010**[a]		−0.8284		−0.2796		−0.1111

Statistical significance results (*P* values only) from separate ANOVA's and ANCOVAS's for circulating hormone levels. Mean hormone concentrations for each diet group are reported in Fig. 2. Categorical factors of diet (Western vs. standard) or line (3 separate HR lines), and covariate of total body fat (see Materials and methods) measured in grams at the end of the experiment were included. Monocyte chemotactic protein-1 (MCP-1), Gastric inhibitory polypeptide (GIP). All *P* values are 2-tailed. Significant main effect values ($P<0.05$) are in bold. Fat mass had a positive and statistically significant effect for the first 3 hormones, but a negative and non-significant effect for the latter 3.
[a] Indicates direction Western diet > standard diet.

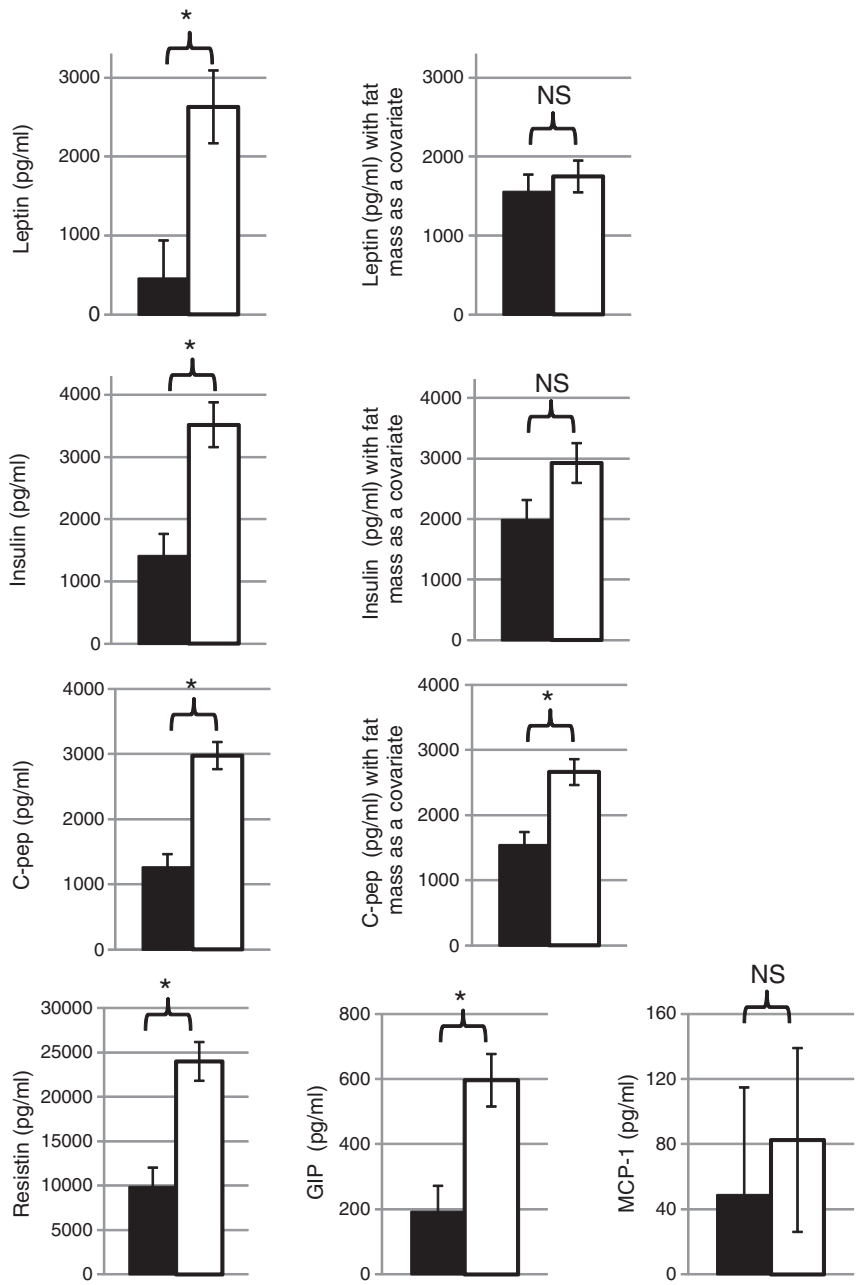

Fig. 2. Least Squares Means ± S.E. for circulating hormone concentrations (pg/ml). Solid bars represent mice fed standard diet and open bars represent mice fed Western diet. Blood samples for the control line were not run, so all data are averages of the three HR lines. Figures with fat mass as a covariate for resistin, monocyte chemotactic protein-1 (MCP-1), and gastric inhibitory polypeptide (GIP) are omitted because the covariate is non-significant. * indicates $P<0.05$. See Table 1 for additional statistical results.

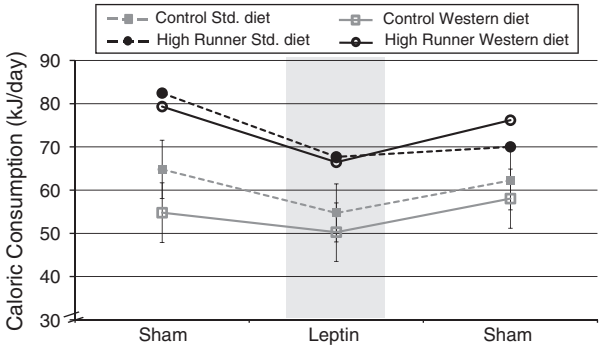

Fig. 3. Least Squares Means for food consumption from repeated-measures analysis of covariance in SAS, using all four lines of mice. Body mass was used as a covariate. Pre-leptin "sham" indicates the average food consumption for the two days of sham injections preceding leptin treatment. "Leptin" is the average food consumption for the three leptin injection days, and post-leptin "sham" is the average food consumption for the final five days of sham injections. The three HR lines were averaged from the analysis with line as a main affect and, therefore, do not have S.E. bars (see Materials and methods).

addition of caloric intake as a covariate, the time × line interaction becomes non-significant ($P = 0.0856$). In a separate analysis of the control line, wheel running was not statistically affected by either leptin treatment or diet, nor their interaction ($P = 0.1896$, 0.0671, 0.1434, respectively).

4. Discussion

Evidence concerning leptin's effect on physical activity has been varied. Leptin injections (I.P.) in mice lacking a functional leptin gene (ob/ob mice) can lead to increases in locomotor activity in a novel cage over 15 min [41] and in voluntary wheel running over a 24-hour period [18]. Intracerebroventricular leptin-injected rats increase spontaneous physical activity during the initial 20 h after injections [42], as well as throughout 5 days of consecutive treatment [17]. In contrast, leptin treatment (I.P. injections or subcutaneous osmotic pumps) in fed wildtype C57Bl/6 mice produced no change in activity in a new cage environment as measured by human observation over 15 min [41] or in wheel running during 4–6 days [18]. Conversely, leptin treatment via minipumps can reduce fasting-induced hyperactivity in Wistar rats and C57Bl/6 mice [18,43].

Several lines of evidence suggest a link between voluntary wheel running, leptin, and effects of a Western diet in HR mice (from lines

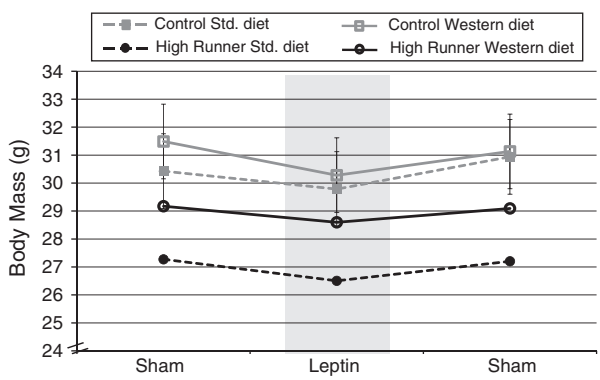

Fig. 4. Least Squares Means for body mass from repeated-measures analysis in SAS, using all four lines of mice. Pre-leptin "sham" indicates the average body mass for the two days of sham injections preceding leptin treatment. "Leptin" is the average body mass for the three leptin injection days, and post-leptin "sham" is the average body mass for the final five days of sham injections. The three HR lines were averaged from the analysis with line as a main effect and, therefore, do not have S.E. bars (see Materials and methods).

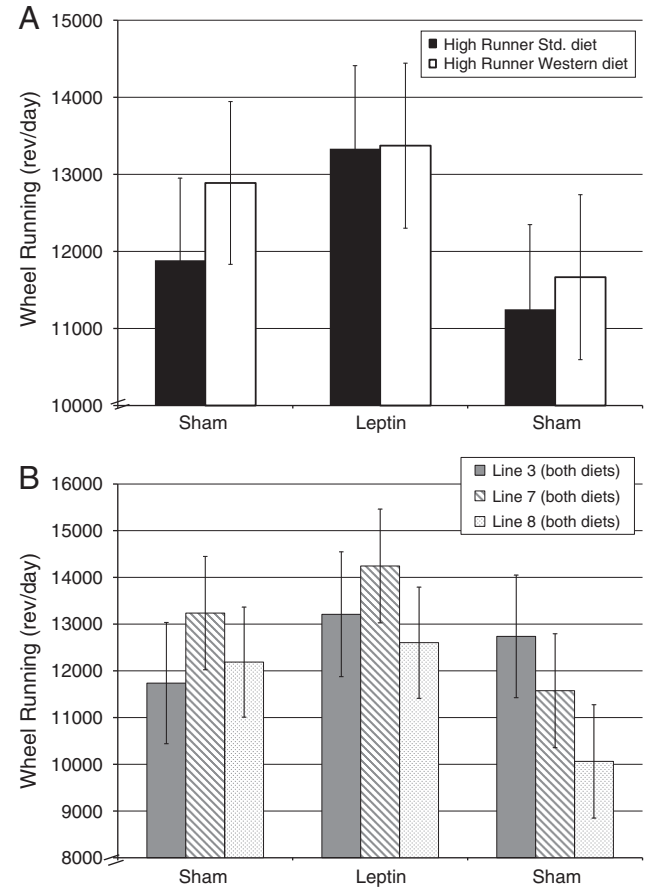

Fig. 5. Least Squares Means ± S.E for wheel running from repeated-measures analysis of the three HR lines. Pre-leptin "sham" indicates the average wheel running for the two days of sham injections preceding leptin treatment. "Leptin" is the average wheel running for the three leptin injection days, and post-leptin "sham" is the average wheel running for the final five days of sham injections. A: Wheel running revolutions for HR mice for each diet. Leptin treatment significantly increased running compared to either the preceding or following shams, but the effect of diet was not statistically significant (see text). B: Wheel running for HR mice from each line (pooled by diet). Leptin treatment significantly increased running compared to either the preceding or following sham injections for all three HR lines, but their response differed slightly (time × line interaction $P = 0.0308$).

selectively bred for high voluntary wheel running). Unlike all pharmacological agents tested thus far in HR mice [28–30,33,44] WD can substantially increase wheel running in males [27] (females were not studied). In the present study, WD increased leptin concentrations nearly 6-fold after only 18 days in male HR mice (Fig. 2) (control lines not assayed). Additionally, acute leptin injections increased wheel running in HR mice (Fig. 5). From these results, it is tempting to deduce that Western diet stimulates wheel running in HR lines at least partly through leptin signaling.

Consistent with this interpretation, leptin injections in HR mice on standard diet produced a larger increase in running (1447 additional revolutions per day) than in HR animals on WD (484 additional revolutions per day [Fig. 5]). However, we did not observe a statistically significant time-by-diet interaction in analysis of HR mice, so it is difficult to conclude leptin injections differentially affected wheel running in the different diet groups.

Similar to the effects observed in inbred strains of rodents on ad lib food [18], leptin injections did not increase running in our non-selected control line of mice. Although leptin was not quantified in mice from the non-selected control line in the present study, previous work has shown that circulating leptin concentrations in HR and control lines respond similarly to changes in fat mass accumulated through aging or Western diet [25,26]. WD increased body mass in

both linetypes in our study, but did not alter wheel-running distances in control mice, nor did leptin injections, so it does not appear that baseline leptin constrains wheel-running activity in wildtype animals. However, HR mice on the standard diet (and WD) significantly increased wheel running with leptin treatment. Thus, a fundamental difference may have evolved between the control and HR lines in this respect. The increased running in HRs due to leptin treatment begs the question as to why HR mice have evolved lower leptin levels in the face of continuous selective breeding for high voluntary wheel running (while they have ad lib access to food).

One reason for increased running may be that low leptin serves as a starvation signal, leading to increased motivation to forage. Fasting in rodents can result in substantial increases in wheel running [9]. During fasting, leptin drops precipitously, contributing to the increase in locomotor activity [18,43]. It seems unlikely, though, that HR mice are running more because their innately low leptin operates as a starvation signal that results in greater motivation for foraging, even though they are wheel-tested with ad lib access to food [43]. If this were the case, then Western diet's effect of increasing leptin (Fig. 2) should have led to decreased wheel running.

Several other explanations are possible for the evolution of low circulating leptin levels in HR mice, including pleiotropic gene action, gene linkage and reward substitution. Some evidence indicates pleiotropic effects of leptin on the central nervous system, which could help explain our results. A potential site for this effect may exist in the hypothalamus, specifically the arcuate nucleus, which is known to be a major regulator in the control of feeding in response to leptin signaling [45]. This region has also recently been implicated in the control of voluntary locomotion. Ambulatory activity in home cages of leptin receptor-deficient mice is normalized after unilateral restoration of leptin receptors in the ARC [45]. Furthermore, signal transducer and activator of transcription 3 (stat3) signaling in Agouti-related protein (AgRP) neurons within the ARC are known to be downstream of leptin signaling. Work with Cre-mediated recombination resulting in constitutively active stat3 signaling in AgRP neurons increased home-cage locomotor activity (as measured by transmitters in the peritoneal cavity) independently of AgRP expression and changes in food intake [46]. If higher levels of circulating leptin are involved in greater locomotor activity of HR mice through signaling in the ARC, but lower leptin is required to stimulate feeding to maintain energy balance, then high levels of voluntary exercise could be constrained by these mechanisms.

In regards to reward substitution, the low body fat (and circulating leptin) in HR mice may result from neural reward generated by high wheel running substituting for the reward that is generated by eating. Reward substitution has been observed in rats, where a preference for high fat diet (over standard chow) in sedentary conditions is eliminated when wheel access is granted [47]. A similar scenario has also been found with nicotine and food consumption in mice. Nicotine activation of pro-opiomelanocortin neurons leads to reduced food intake and subsequently reduced body mass [48]. If there is a parallel relationship in HR mice between exercise and food consumption, then the independent evolution of a smaller body size in all four HR lines could, at least in part, be a consequence of pleasure generated from running. Alternatively, it has been suggested that the reduced body size of HR mice may be related to their elevated circulating corticosterone levels [49,50]. An additional hypothesis, suggested by Plomin and colleagues [51] (pp. 261–262 and their Fig. 10.1), is that perturbations in tyrosine hydroxylase or dopa decarboxylase enzymes could affect dopamine and/or norepinephrine simultaneously and result in more wheel running and less eating.

Recent evidence supports the reward substitution hypothesis, as HR mice, when switched from tap water to a non-caloric sweet drink (Sweet 'N Low, Equal or Splenda) do not increase consumption to the same extent as controls [52]. "This alteration in incentive salience for a competing reward is characteristic of reward substitution, where a highly valued reward (wheel running) substitutes or obscures the effect of a competing reward (sweet taste)…" [52] (p.13).

With the exception of monocyte chemotactic protein-1 (MCP-1), involved in inflammation, WD increased all the measured hormone concentrations. Interestingly, body fat, which was measured one month after the blood samples were taken, was still positively correlated with several hormone concentrations in the ANCOVA models (Table 1). Both gastric inhibitory polypeptide (GIP) and bioactive molecule C-peptide are involved in insulin secretion and are elevated, along with insulin, in mice fed WD. These correlations suggest the development of insulin resistance. There is also evidence of greater systemic inflammation, as resistin concentrations rose 2.4-fold in the WD group (although MCP-1 did not significantly increase). Thus, when given WD for several weeks, the HR mice appear to develop many of the same metabolic abnormalities as a typical mouse would, despite their high running. Some of these changes are closely related to changes in fat mass, while others, like increases in resistin and GIP, are not.

It is well known that high leptin concentrations can cause leptin resistance [53,54]. Lin et al. [54] showed that one week of WD did not affect peripheral leptin sensitivity but eight weeks of WD in sedentary conditions was enough to induce resistance to exogenous leptin administration. In our study, animals were on Western diet for 37 days before leptin injections began. WD mice gained considerable fat mass and had corresponding high leptin. However, WD mice do not appear to have developed leptin resistance. In terms of food consumption and body mass changes, all groups responded to leptin treatment to a similar degree (Figs. 3 and 4). It is possible that not enough time elapsed for mice to develop leptin resistance. Importantly, all of the mice in our study had access to wheels, which could be helping animals maintain central leptin sensitivity [47].

Over the course of 61 generations of selective breeding for high voluntary wheel running, mice have evolved lower body size, low plasma leptin concentrations, and neurological differences related to motivation for wheel running. Despite the evolution of low circulating leptin levels, exogenous leptin treatment increases wheel running in male HR mice but not in males from a non-selected control line. However, it remains unclear if WD elevates running in HR mice through increases in leptin concentration. Understanding the physiological mechanisms underlying these effects will provide insight into the genetics and evolution of voluntary behaviors and overall energetic homeostasis.

Acknowledgments

We would like to thank Gerald C. Claghorn, Paul Chan, and Genesis Ordonez for assistance. Supported by University of California, Riverside Undergraduate Research Award to KTV and NSF grant IOS-1121273 to TG.

References

[1] Garland Jr T, Schutz H, Chappell MA, Keeney BK, Meek TH, Copes LE, et al. The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. J Exp Biol 2011;214:206–29.
[2] Tokuyama K, Saito M, Okuda H. Effects of wheel running on food intake and weight gain of male and female rats. Physiol Behav 1982;28:899–903.
[3] Bell RR, Spencer MJ, Sherriff JL. Voluntary exercise and monounsaturated canola oil reduce fat gain in mice fed diets high in fat. J Nutr 1997;127:2006–10.
[4] Koteja P, Swallow JG, Carter PA, Garland Jr T. Energy cost of wheel running in house mice: implications for coadaptation of locomotion energy budgets. Physiol Biochem Zool 1999;72:238–49.
[5] Swallow JG, Koteja P, Carter PA, Garland Jr T. Food consumption and body composition in mice selected for high wheel-running activity. J Comp Physiol B 2001;171:651–9.
[6] Jung AP, Luthin DR. Wheel access does not attenuate weight gain in mice fed high-fat or high-CHO diets. Med Sci Sports Exerc 2010;42:355–60.

[7] Jung AP, Curtis TS, Turner MJ, Lightfoot JT. Physical activity and food consumption in high- and low-active inbred mouse strains. Med Sci Sports Exerc 2010;42:1826–33.
[8] Overton JM, Williams TD. Behavioral and physiologic responses to caloric restriction in mice. Physiol Behav 2004;81:749–54.
[9] Adan RAH, Hillebrand JJG, Danner UN, Cardona Cano S, Kas MJH, Verhagen LAW. Neurobiology driving hyperactivity in activity-based anorexia. Current Topics in Behavioral Neurosciences 2010;6:229–50.
[10] Brownlow BS, Petro A, Feinglos MN, Surwit RS. The role of motor activity in diet-induced obesity in C57BL/6J mice. Physiol Behav 1996;60:37–41.
[11] Novak CM, Kotz CM, Levine JA. Central orexin sensitivity, physical activity, and obesity in diet-induced obese and diet-resistant rats. Am J Physiol Endocrinol Metab 2006;290:E396–403.
[12] Bjursell M, Gerdin A-K, Lelliott CJ, Egecioglu E, Elmgren A, Tornell J, et al. Acutely reduced locomotor activity is a major contributor to Western diet-induced obesity in mice. Am J Physiol Endocrinol Metab 2008;294:E251–60.
[13] Simoncic M, Horvat S, Stevenson PL, Bunger L, Holmes MC, Kenyon CJ, 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.
[14] Baile CA, Della-Fera MA, Martin RJ. Regulation of metabolism and body fat mass by leptin. Annu Rev Nutr 2000;20:105–27.
[15] Karsenty G. Convergence between bone and energy homeostases: leptin regulation of bone mass. Cell Metab 2006;4:341–8.
[16] Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature 2006;443:289–95.
[17] Choi Y-H, Li CL, Hartzell DL, Little DE, Della-Fera MA, Baile CA. ICV leptin effects on spontaneous physical activity and feeding behavior in rats. Behav Brain Res 2008;188:100–8.
[18] Morton GJ, Kaiyala K, Fisher JD, Ogimoto K, Schwartz MW, Wisse BE. Identification of a physiological role for leptin in the regulation of ambulatory activity and wheel running in mice. Am J Physiol Endocrinol Metab 2011;300:E392–401.
[19] Levine JA. Nonexercise activity thermogenesis (NEAT): environment and biology. Am J Physiol Endocrinol Metab 2004;286:675–85.
[20] Levine JA, Lanningham-Foster LM, McCrady SK, Krizan AC, Olson LR, Kane PH, et al. Interindividual variation in posture allocation: possible role in human obesity. Science 2005;307:584–6.
[21] Nehrenberg DL, Hua K, Estrada-Smith D, Garland Jr T, Pomp D. Voluntary exercise and its effects on body composition depend on genetic selection history. Obesity 2009;17:1402–9.
[22] Swallow JG, Rhodes JS, Garland Jr T. Phenotypic and evolutionary plasticity of organ masses in response to voluntary exercise in house mice. Integr Comp Biol 2005;45:426–37.
[23] Garland Jr T, Kelly SA, Malisch JL, Kolb EM, Hannon RM, Keeney BK, et al. How to run far: multiple solutions and sex-specific responses to selective breeding for high voluntary activity levels. Proc Biol Sci 2011;278:574–81.
[24] Girard I, Rezende EL, Garland Jr T. Leptin levels and body composition of mice selectively bred for high voluntary activity. Physiol Biochem Zool 2007;80:568–79.
[25] Vaanholt LM, Jonas I, Doornbos M, Schubert KA, Nyakas C, Garland Jr T, et al. Metabolic and behavioral responses to high-fat feeding in mice selectively bred for high wheel-running activity. Int J Obes 2008;32:1566–75.
[26] Vaanholt LM, Meerlo P, Garland Jr T, Visser GH, van Dijk G. Plasma adiponectin is increased in mice selectively bred for high wheel-running activity, but not by wheel running per sé. Horm Metab Res 2007;39:377–83.
[27] Meek TH, Eisenmann JC, Garland Jr T. Western diet increases wheel running in mice selectively bred for high voluntary wheel running. Int J Obes 2010;34:960–9.
[28] Rhodes JS, Hosack GR, Girard I, Kelley AE, Mitchell GS, Garland Jr T. Differential sensitivity to acute administration of cocaine GBR 12909, and fluoxetine in mice selectively bred for hyperactive wheel-running behavior. Psychopharmacology 2001;158:120–31.
[29] Rhodes JS, Garland Jr T. Differential sensitivity to acute administration of Ritalin, apormorphine, SCH 23390, but not raclopride in mice selectively bred for hyperactive wheelrunning behavior. Psychopharmacology 2003;167:242–50.
[30] Rhodes JS, Gammie SC, Garland Jr T. Neurobiology of mice selected for high voluntary wheel-running activity. Integr Comp Biol 2005;45:438–55.
[31] Rhodes JS, Garland Jr T, Gammie SC. Patterns of brain activity associated with variation in voluntary wheel-running behavior. Behav Neurosci 2003;117:1243–56.
[32] Hommel JD, Trinko R, Sears RM, Georgescu D, Liu Z-W, Gao X-B. Leptin receptor signaling in midbrain dopamine neurons regulates feeding. Neuron 2006;51:801–10.
[33] Keeney BK, Raichlen DA, Meek TH, Wijeratne RS, Middleton KM, Gerdeman GL, et al. Differential response to a selective cannabinoid receptor antagonist (SR141716: rimonabant) in female mice from lines selectively bred for high voluntary wheel-running behavior. Behav Pharmacol 2008;19:812–20.
[34] Swallow JG, Carter PA, Garland Jr T. Artificial selection for increased wheel-running behavior in house mice. Behav Genet 1998;28:227–37.
[35] Koteja P, Carter PA, Swallow JG, Garland Jr T. Food wasting by house mice: variation among individuals, families, and genetic lines. Physiol Behav 2003;80:375–83.
[36] Cinti S. The adipose organ. Prostaglandins Leukot Essent Fatty Acids 2005;73:9–15.
[37] Garland Jr T, Morgan MT, Swallow JG, Rhodes JS, Girard I, Belter JG, et al. Evolution of a small-muscle polymorphism in lines of house mice selected for high activity levels. Evolution 2002;56:1267–75.
[38] Houle-Leroy P, Garland Jr T, Swallow JG, Guderley HP. Artificial selection for high activity favors mighty mini-muscles in house mice. Am J Physiol Regul Integr Comp Physiol 2003;284:R433–43.
[39] Bilodeau GM, Guderley H, Joanisse DR, Garland Jr T. Reduction of type IIb myosin and IIB fibers in tibialis anterior muscle of mini-muscle mice from high-activity lines. J Exp Zool 2009;311A:189–98.
[40] Syme DA, Evashuk K, Grintuch B, Rezende EL, Garland Jr T. Contractile abilities of normal and "mini" triceps surae muscles from mice (*Mus domesticus*) selectively bred for high voluntary wheel running. J Appl Physiol 2005;99:1308–16.
[41] Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995;269:540–6.
[42] Ainslie DA, Morris MJ, Wittert G, Turnbull H, Proietto J, Thorburn AW. Estrogen deficiency causes central leptin insensitivity and increased hypothalamic neuropeptide Y. Int J Obes 2001;25:1680–8.
[43] Exner C, Hebebrand J, Remschmidt H, Wewetzer C, Ziegler A, Herpertz S, et al. Leptin suppresses semi-starvation induced hyperactivity in rats: implications for anorexia nervosa. Mol Psychiatry 2000;5:476–81.
[44] Li G, Rhodes JS, Girard I, Gammie SC, Garland Jr T. Opioid-mediated pain sensitivity in mice bred for high voluntary wheel running. Physiol Behav 2004;83:515–24.
[45] Coppari R, Ichinose M, Lee CE, Pullen AE, Kenny CD, McGovern RA, et al. The hypothalamic arcuate nucleus: a key site for mediating leptin's effects on glucose homeostasis and locomotor activity. Cell Metab 2005;1:63–72.
[46] Mesaros A, Koralov SB, Rother E, Wunderlich FT, Ernst MB, Barsh GS, et al. Activation of stat3 signaling in AgRP neurons promotes locomotor activity. Cell Metab 2008;7:236–48.
[47] Scarpace PJ, Matheny M, Zhang Y. Wheel running eliminates high-fat preference and enhances leptin signaling in the ventral tegmental area. Physiol Behav 2010;100:173–9.
[48] Mineur YS, Alfonso A, Rao Y, Salas R, DiLeone RJ, Gundisch D, et al. Nicotine decreases food intake through activation of POMC neurons. Science 2011;332:1330–2.
[49] Girard I, Garland Jr T. Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. J Appl Physiol 2002;92:1553–61.
[50] Malisch JL, Saltzman W, Gomes FR, Rezende EL, Jeske DR, Garland Jr T. Baseline and stress-induced plasma corticosterone concentrations of mice selectively bred for high voluntary wheel running. Physiol Biochem Zool 2007;80:146–56.
[51] Plomin R, DeFries JC, McClearn GE. Behavioral genetics: a primer. 2nd ed. New York: WH Freeman; 1990.
[52] Kolb EM. 2010. Neurobiological and physiological underpinnings of high voluntary wheel running. (Doctoral dissertation). University of California, Riverside. Retrieved from ProQuest Dissertations and Theses. (Accession Order No. AAT 3426141)
[53] El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. J Clin Invest 2000;105:1827–32.
[54] Lin S, Thomas TC, Storlien LH, Huang XF. Development of high fat diet-induced obesity and leptin resistance in C57B1/6J mice. Int J Obes 2000;24:639–46.