Physiology & Behavior 139 (2015) 303–313

Contents lists available at ScienceDirect

Physiology & Behavior

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

Physically active rats lose more weight during calorie restriction

Mark E. Smyers [a,b,*], Kailey Z. Bachir [c], Steven L. Britton [d], Lauren G. Koch [d], Colleen M. Novak [a,b]

[a] School of Biomedical Sciences, Kent State University, Kent, OH, USA
[b] Department of Biological Sciences, Kent State University, Kent, OH, USA
[c] College of Health Sciences, Cleveland State University, Cleveland, OH, USA
[d] Department of Anesthesiology, University of Michigan, Ann Arbor, MI, USA

HIGHLIGHTS

- High intrinsic aerobic fitness is linked to favorable metabolic health.
- Rats selected for high intrinsic aerobic capacity (HCR) are more physically active.
- HCR lose more body weight under calorie restriction.
- HCR also remain more physically active during calorie restriction.
- Metabolic thriftiness and defense of body weight are linked to low aerobic capacity.

ARTICLE INFO

Article history:
Received 11 July 2014
Received in revised form 4 November 2014
Accepted 5 November 2014
Available online 20 November 2014

Keywords:
High- and low-capacity runners (HCR and LCR)
Weight loss
Thrifty genotype
Non-exercise activity thermogenesis (NEAT)

ABSTRACT

Daily physical activity shows substantial inter-individual variation, and low physical activity is associated with obesity and weight gain. Elevated physical activity is also associated with high intrinsic aerobic capacity, which confers considerable metabolic health benefits. Rats artificially selected for high intrinsic aerobic capacity (high-capacity runners, HCR) are more physically active than their low-capacity counterparts (low-capacity runners, LCR). To test the hypothesis that physical activity counters metabolic thriftiness, we measured physical activity and weight loss during three weeks of 50% calorie restriction (CR) in the HCR and LCR rat lines. At baseline, HCR ate more and were more active than LCR; this was seen in male rats, where LCR are considerably heavier than HCR, as well as in a set of female rats where body weight did not differ between the lines, demonstrating that this effect is consistent across sex and not secondary to body weight. We show for the first time that HCR lose more weight than LCR relative to baseline. Physical activity levels declined throughout CR, and this was more pronounced in HCR than in LCR, yet some aspects of activity remained elevated in HCR relative to LCR even during CR. This is consistent with the idea that low physical activity contributes to metabolic thriftiness during food restriction, allowing LCR to defend body mass, particularly lean mass. This has implications for physical activity during diet-induced weight loss, the genetic underpinnings of individual differences in weight loss during a diet, and the potential evolutionary opposition between metabolic thriftiness and aerobic capacity.

1. Introduction

Weight gain and obesity result from low energy expenditure for the level of energy intake. The negative influence of obesity in a host of medical conditions underscores the need to understand what underlies individual differences in obesity susceptibility. Even in an obesogenic environment, however, about a third of our adult population remains lean [1]. The energy expended in the activities of daily living, also known as non-exercise activity thermogenesis (NEAT), confers protection against fat gain during overfeeding in humans [2]. Daily physical activity and NEAT are biologically regulated, heritable traits [3–5], but relatively little is known regarding the mechanisms underlying the tendency to be physically active, or how these may change under energetically challenging conditions [reviewed in 4,6,7]. While descriptive accounts have noted that severe energy restriction (~50% for 24 weeks) results in lethargy and suppressed physical activity in people [8], varied methodologies have resulted in different answers regarding how food restriction affects physical activity in animals and humans [9–12].

Physical activity fosters success in weight loss and maintenance of metabolic health, while low activity predicts negative metabolic

Abbreviations: ANOVA, analysis of variance; BW, body weight; CR, calorie restriction; HCR, high-capacity runners; NEAT, non-exercise activity thermogenesis; LCR, low-capacity runners.

* Corresponding author at: School of Biomedical Sciences, Kent State University, P.O. Box 5190, A328/A330 Cunningham Hall, Kent, OH 44242, USA. Tel.: +1 330 672 2304; fax: +1 330 670 3713.
E-mail address: msmyers1@kent.edu (M.E. Smyers).

http://dx.doi.org/10.1016/j.physbeh.2014.11.044
0031-9384/

outcomes [13–19]. Like obesity, "spontaneous" or daily physical activity has a strong hereditary component [3–5,20–24]. We have found a strong association between levels of daily physical activity and intrinsic aerobic capacity [25–27]. In both humans and rats, individuals with high exercise capacity are consistently more physically active and resistant to metabolic and cardiovascular disease [25–31]. Based on the hypothesis that aerobic capacity shapes the vulnerability to complex disease (the aerobic hypothesis), a rat model system was developed through divergent artificial selection for intrinsic aerobic capacity, resulting in high- and low-capacity runners (HCR and LCR) derived from a genetically heterogeneous founder population [32]. For selection based on phenotype, aerobic capacity was assessed in the founder population and subsequent generations by determining maximal treadmill running endurance: the male and female from each litter with the best (i.e., longest) running time were selected for breeding using an algorithm to minimize inbreeding; the same was done for rats with the shortest running time. Aerobic capacity was measured in offspring at 3 months of age, and the phenotype has been continually refined at each generation. The result is rats with intrinsically high and low aerobic capacity, independent of training, with associated differences in cardiovascular and metabolic disease factors [33]. Importantly, in the selection process, low NEAT segregated with low aerobic capacity, and high NEAT with high aerobic capacity [25–27]. The active rats are also resistant to obesity and metabolic disease [32,33]. This allows us to investigate the mechanisms underlying high NEAT, specifically to test the hypothesis that obesity or the tendency to become obese is related to an increased "thriftiness" of metabolism [34].

People differ not only in obesity propensity but also in their ability to lose weight on a diet [35,36]; the same is true for laboratory animals [37]. Apart from the known importance of diet adherence [36], these differences may be attributable to disparities in energy expenditure, reflecting differences in metabolic "thriftiness" during food restriction [10,35]. Activities of daily living may contribute to differences in energy expenditure and weight loss during calorie restriction (CR) [10,35,36]. If the trade-off for elevated intrinsic aerobic capacity is a compromise in fuel efficiency or "thriftiness," then those with high aerobic capacity and physical activity would lack the metabolic thriftiness necessary to conserve energy and body fat during food scarcity. For example, obese rats lacking leptin receptors are resistant to starvation [38,39]. To test this hypothesis in relation to physical activity and aerobic capacity, we assessed the ability of alterations in behavior—elevated physical activity—to promote negative energy balance and, therefore, weight loss, as well as the impact of decreased food availability on physical activity levels.

2. Methods

2.1. Ethical approval

Animal protocols were approved by the Kent State University Institutional Animal Care and Use Committee.

2.2. Diet

Food (5P00 MRH 3000, T.R. Last Co., Inc.) was available ad libitum except during calorie restriction. Prolab RMH 3000 Chow Pellets are comprised of 26% protein, 14% porcine and plant oil fat, and 60% carbohydrate, with a physiological fuel value of 3.46 kcal/g. Consideration of the risk for inadequacy of nutrients is important, especially when macronutrients are varied for specific weight loss diets [40]. The micronutrients of this chow are approximately 3 times the required micronutrient levels for maintenance/growth of rats [41] so it can be considered a nutrient-dense source of macronutrients. Thus the 50% gram reduction of food that was utilized in our studies restricts the number of available calories but still meets the minimum micronutrient requirements in adult rats for preventing malnutrition especially for the three-week duration of our calorie restriction paradigm. In addition, rats were carefully monitored for behavioral signs of nutritional deficiencies with none noted other than an increase in anxiety in many of the HCR and an expected weight loss in both strains. HCR and LCR have been previously subjected to long-term (3 months to 12 months) calorie restrictions at 30% of a similar standard rat chow (Lab Diet no. 5001) with no discernable effects of malnutrition being reported [42].

2.3. Animals

HCR and LCR rats from the University of Michigan were used for these studies. Rats were housed on a 12:12 light:dark cycle, with lights on at 0700 Eastern Standard Time, and with ad libitum water availability at all times. Rats that underwent calorie restriction were from generation 26 and generation 27 and ranged from 10 to 14 months of age; female HCR and LCR overlapped in body weights [25]. Body weight was measured using an Ohaus triple-beam balance with a resolution of 0.5 g, and body composition was measured using an EchoMRI-700 (Echo Medical Systems, Houston, TX) during the mid-light phase. For daily estrus state determination, female rats were first bundled in a cloth, held upside down in one hand, while the other hand gently pipetted 100 μl of saline into the vaginal opening. The rinse solution was then removed with a plastic pipette and placed into a microtube. Approximately 10 μl of this wash was placed on a glass slide and observed with a light microscope. Estrus stage was determined by approximate ratio of cell types as described by Marcondes et al. (2003) [43]. Estrus determination began during the last 3 days of activity monitoring and continued for 8 days after monitoring ceased in order to avoid stress-related alteration of daily activity measures, and cycles were interpolated onto activity records; only rats with clear, robust cycles were considered when relating stages of the estrous cycle with activity levels. Cycles were not measured during or after CR.

Four studies were conducted. First, baseline physical activity and food intake was measured in generation 26 female HCR ($n = 13$) and LCR ($n = 13$) over 14 days. Because of the limited number of activity monitors (16), data for these rats were obtained from 2 cohorts, with an equal representation of HCR and LCR in each cohort. Estrous cycles were monitored at the end of activity measurement in these rats to determine if a rhythm in spontaneous physical activity could be detected, and to determine the magnitude of this rhythm if present. In the second study, baseline physical activity was measured in generation 27 male HCR ($n = 10$) and LCR ($n = 6$) for 7 days. In the third study, the effects of 50% CR on body weight, body composition, and physical activity were evaluated in generation 26 female HCR ($n = 8$) and LCR ($n = 8$). In a separate group of females, the effects of longer-term (5 weeks) CR were measured in a small group of generation 26 HCR ($n = 4$) and LCR ($n = 4$) females, with body composition measured weekly. In the fourth and final study, physical activity and body weight were measured daily during and after 21 days of CR in male HCR ($n = 10$) and LCR ($n = 6$) immediately after baseline activity measurements.

2.4. Activity measurement

Home-cage spontaneous (non-exercise) physical activity was quantified within Plexiglas chambers ($41 \times 41 \times 25$ cm) with an elevated floor grid. Activity was measured using monitors that calculate beam breaks of infrared photocells spaced 2.54 cm apart in the X and Y axes placed 4.5 cm (for horizontal activity) and 13.5 cm (for vertical activity) above the platform (Opto-Varimex 4 Auto-Track system, Columbus Instruments). Long-term continual monitoring of movement occurred except for daily periods of animal maintenance and body weight measurements (1–2 h). Activity measurements included ambulation: distance traveled (cm/min), horizontal beam-break counts, ambulatory beam-break counts (excluded consecutive breaks of the same beam), and time spent ambulating. Second, non-ambulatory activity measures included stationary or stereotypic activity counts (consecutive breaks

of the same beam during repetitive movement such as grooming), time spent in stereotypic movement, and time spent stationary or resting (no beam breaks). Lastly, we calculated vertical beam-breaks and time spent in vertical movement. In a subset of generation 26 rats (6 HCR and 7 LCR), activity was assessed at two different ages (1 year 6 months, and again at 1 year 11 months); distance traveled on the 4th through 9th day in the activity monitor was averaged and compared between groups to measure age-related changes in physical activity in female rats.

2.5. Calorie restriction

For calorie restriction in the females, food intake values over six days of ad lib feeding of rat chow were averaged for each animal during the baseline measurements of food intake and activity levels. Rats were monitored for daily activity distance for 23 additional days during food reduction of 50%, followed by ad lib feeding for 20 days with daily activity being monitored. Activity level determinations were continued during refeeding at ad lib levels for 21 days after completion of

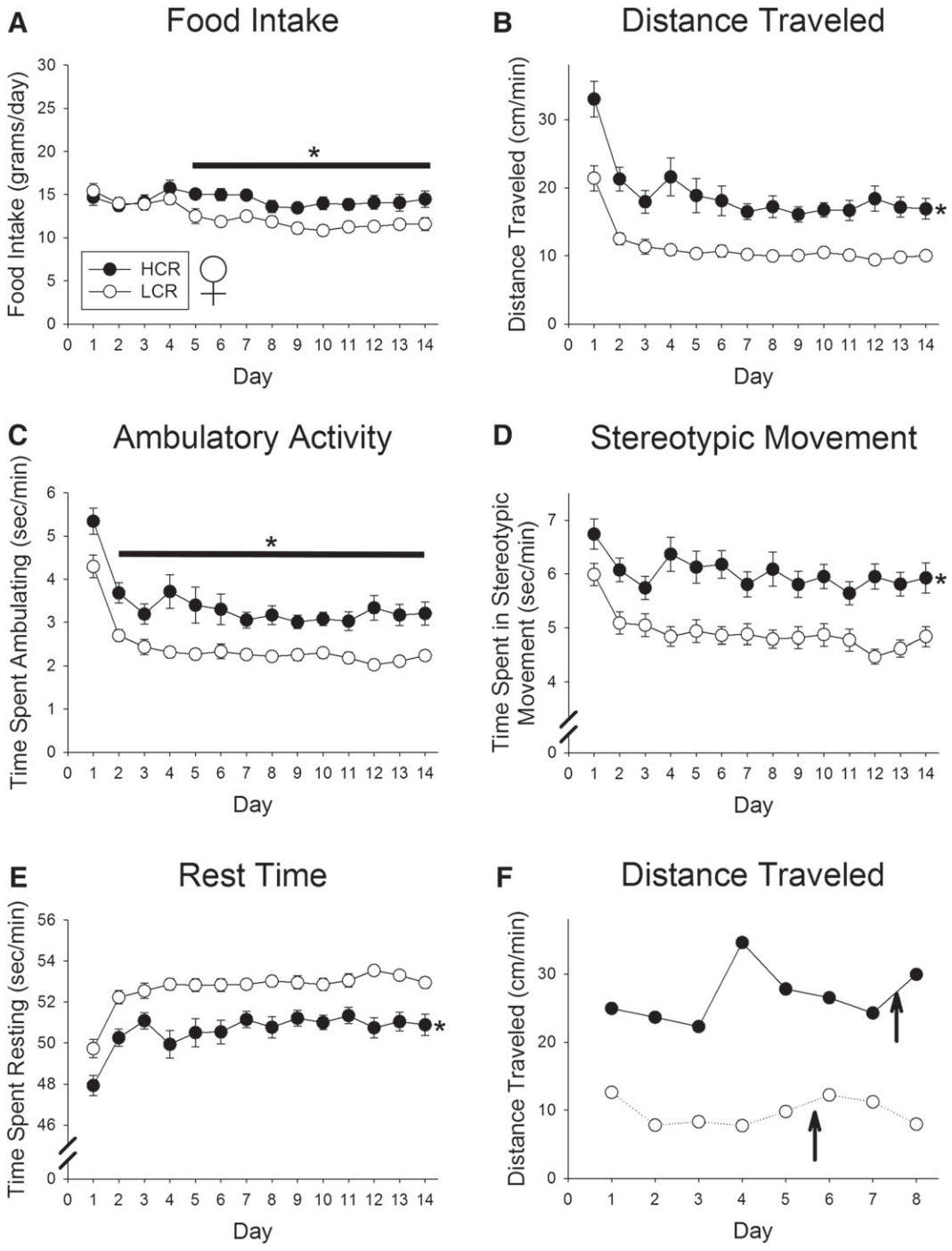

Fig. 1. Food intake and physical activity in female high- and low-capacity rats (HCR and LCR). (A) By the fifth day in the activity monitors, female HCR ate significantly more than female LCR, despite similar body weights. Physical activity including distance traveled (B), time spent ambulating (C), and time spent in stereotypic movement (D) showed similar pattern where all rats showed elevated activity levels upon entering the activity monitors, but normalized within 2–4 days. HCR were consistently more active than LCR in all dimensions of activity investigated throughout activity measurement. (E) Time spent sedentary showed the opposite pattern as physical activity (HCR < LCR), with both groups increasing rest time after the first day in the activity monitor. (F) As illustrated by a representative HCR and LCR, many of the female rats showed detectable estrous cycles, with a low-amplitude but distinguishable 4-to-5-day rhythm in activity in many of these rats. Arrows mark the day prior to the night of estrus. *$p < 0.05$, HCR ≠ LCR on each day unless limitation indicated by line.

CR. Body composition was measured in the mid-light phase on two days prior to the onset of CR, the final day of CR, and after 13–14 days of recovery from CR.

Calorie restriction for males lasted 21 days; 50% CR was calculated as 50% of the average of 6 days ad libitum food intake. Activity was measured throughout the baseline measurement, 21 days of 50% CR, and 14 days of recovery with ad libitum feeding. In all rats, food intake (food provided minus food remaining, including remnants that had fallen through the platform), water intake, and activity were measured daily.

3. Statistical analyses

A mixed-design (split-plot) analysis of variance (ANOVA) was used to analyze physical activity, with line of rat (HCR vs. LCR) as the between-subjects independent variable, and time of measurement as

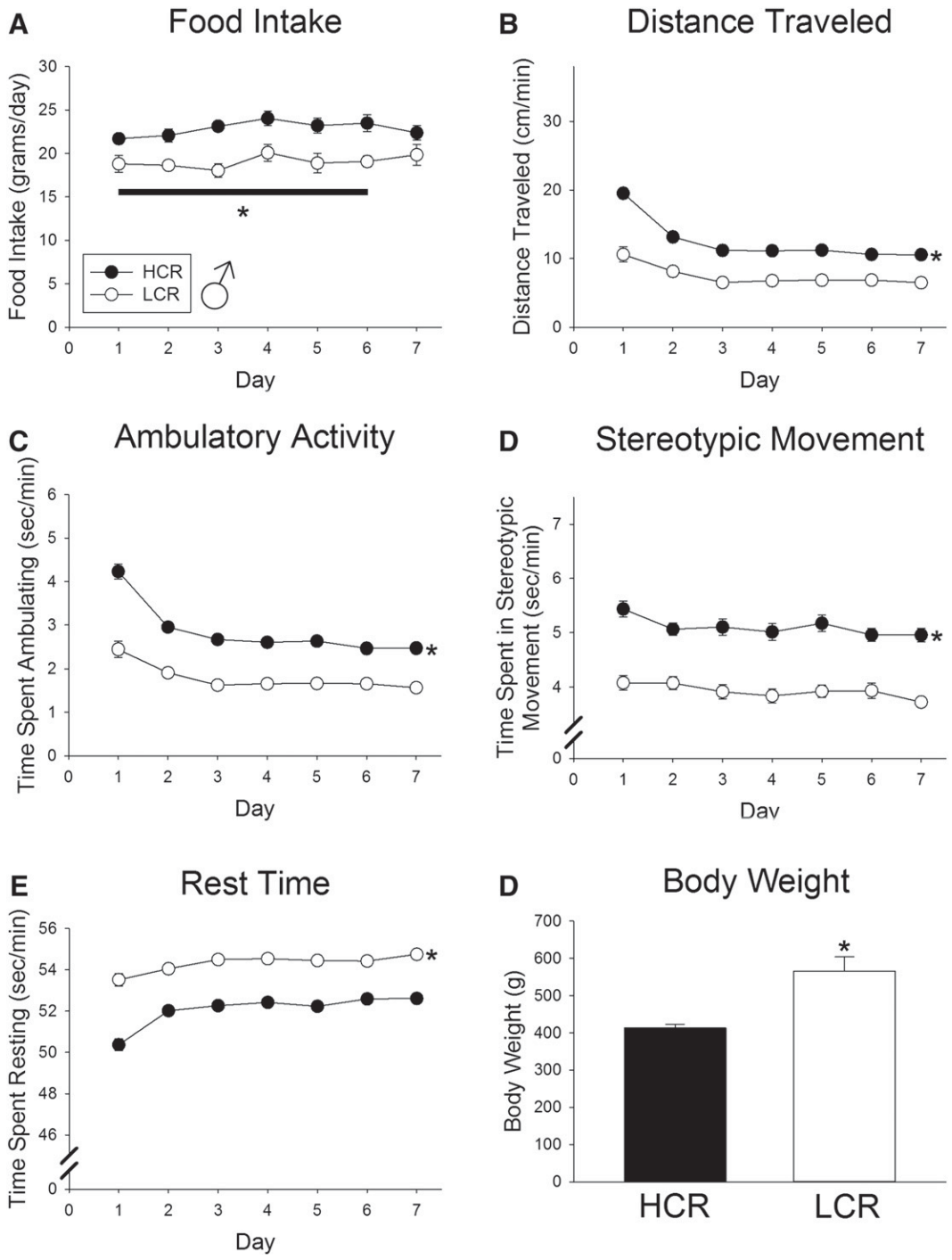

Fig. 2. Food intake, body weight, and physical activity in male high- and low-capacity rats (HCR and LCR). (A) HCR ate more than LCR throughout the time in the activity monitors. HCR were also consistently more active than LCR males, covering more distance per day (B), and spending more time in ambulatory (C) and stereotypic (D) movements, and less time resting (E). Activity was elevated in the first days in the activity monitor relative to subsequent days similarly in both groups of rats, normalizing after 2–3 days. (F) LCR males weighed significantly more than did HCR males. Note that axis scales (A–E) are the same as in Fig. 1. *$p < 0.05$, HCR ≠ LCR on each day unless limitation indicated by line.

the within-subjects independent variable. This was used to analyze physical activity (baseline and during CR), body weight, change in body weight or composition (as a proportion of baseline body weight), and body composition (fat mass in grams, lean mass in grams, percent body fat of total mass, percent lean mass of total mass, percent of baseline fat mass, and percent of baseline lean mass). This analysis was also used to compare activity and body weight of HCR and LCR over different ages. Values at any given time point (e.g., baseline body weights) of HCR and LCR were compared using an unpaired 1-tailed t-test; the 1-tailed test was chosen due to existing data demonstrating elevated activity levels and low body weight in HCR [25–27]. For female vertical activity during CR, daily values were averaged to yield values for before CR, during CR, and after CR. Differences of $p < 0.05$ were considered significant.

4. Results

4.1. Genetically lean female rats showed elevated levels of daily physical activity

This group of female HCR and LCR did not differ in initial body weight (HCR, 266 ± 7 g; LCR, 284 ± 11 g). Female HCR ate more than female LCR (Fig. 1A), with a group by time interaction where a

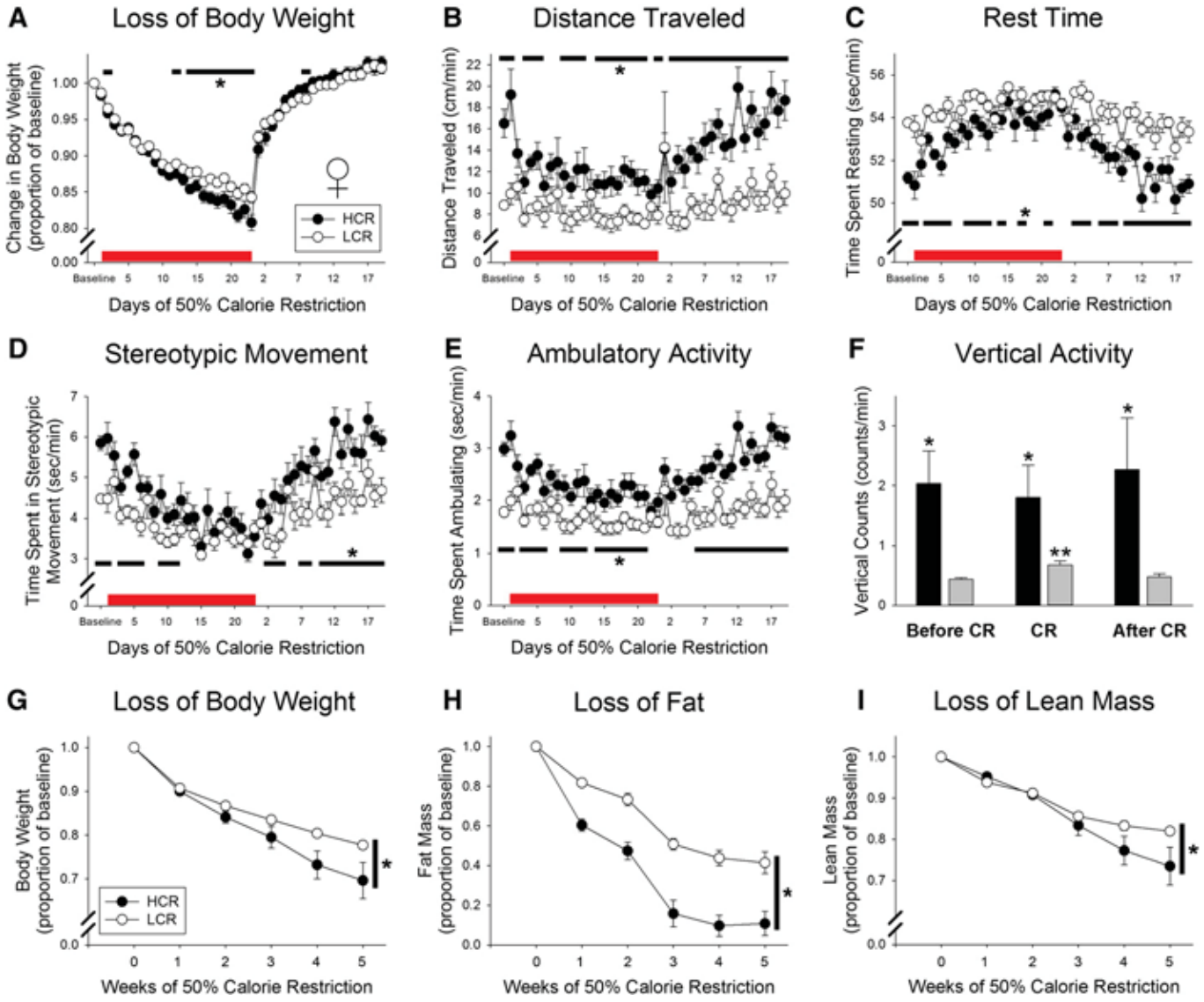

Fig. 3. 50% calorie restriction suppressed physical activity and produced greater weight loss in the more physically active female rats selected for high aerobic capacity (HCR) compared to low-capacity (LCR) females (calorie restriction, CR; 23-day duration represented by line adjacent to X-axis). (A) HCR females lost more weight than did female LCR as a proportion of their baseline body weight; baseline weights did not significantly differ between groups. CR induced a characteristic change in physical activity levels in both groups of female rats; activity levels were elevated on the first 1–2 days of CR, then suppressed over the course of CR. (B) Distance traveled was suppressed by CR, but the effect was more noticeable in female HCR due to their higher baseline activity levels; HCR remained more active than LCR throughout most of CR and recovery. (C) the opposite pattern was seen in time spent resting, where HCR females reached levels equivalent to LCR during the latter half of CR. Whereas stereotypic movement (D) was also suppressed to LCR-like levels after less than 2 weeks of CR, ambulatory activity (E) remained elevated in the female HCR relative to female LCR on most days of CR. (F) CR also affected vertical activity differently in HCR and LCR females; HCR females showed more vertical activity than did LCR females, but whereas vertical activity did not significantly change over CR in HCR, vertical counts significantly increased during CR in LCR. In all other dimensions of physical activity (B–E), activity gradually returned to pre-CR levels after resumption of ad-lib feeding. In a separate group of female rats, body composition was measured weekly for 5 weeks of CR (*significant interaction, G–I). (G) As before, female HCR lost a greater proportion of their baseline body weight during CR. This was due to the HCR females losing more fat mass (H), especially in the first 3 weeks of CR, as well as more lean mass (I), mostly in the 4th and 5th weeks of CR. *HCR ≠ LCR (on days indicated by horizontal line, A–F); **different from before and after CR.

Table 1
Body weight and composition before and after 50% calorie restriction (CR) and after ad-lib recovery, in male and female high- and low-capacity rats (HCR, LCR).

		Baseline					Calorie restriction	
		BW	Fat mass	Lean mass	%Fat	%Lean	BW	Fat mass
Female	HCR	**231.08** ± 5.86	**19.2** ± 1.11	**178** ± 4.83	**8.34** ± 0.49	**77.18** ± 0.51	**187.01** ± 5.34*	**5.00** ± 1.95
	LCR	**228.20** ± 5.41	**25.2** ± 1.57	**168** ± 4.36	**11.03** ± 0.62	**73.58** ± 0.51	**192.08** ± 4.87	**12.31** ± 1.75
	HCR ≠ LCR (p-value)	0.3619	**0.004**	0.066	**0.002**	<**0.001**	0.248	**0.007**
Male	HCR	**414.80** ± 9.89	**57.36** ± 6.24	**295.88** ± 7.19	**13.70** ± 1.35	**71.43** ± 1.21	**340.40** ± 9.44*	**32.29** ± 5.39*
	LCR	**593.08** ± 41.56	**172.10** ± 16.22	**342.32** ± 21.59	**28.81** ± 0.95	**57.90** ± 0.95	**503.25** ± 37.99	**114.45** ± 12.25
	HCR ≠ LCR (p-value)	<**0.001**	<**0.001**	**0.014**	<**0.001**	<**0.001**	<**0.001**	<**0.001**

Body weight (BW) at time of body composition measurement (Regain = after 19 or 14 days of ad libitum feeding for females and males, respectively). Significant p-values are bolded.
* Significantly different change between baseline and CR, or between CR and regain, compared to LCR (1-tailed t-test).

significant difference arose between phenotypes only by the 5th day of activity measurement. For water intake, one outlier LCR was removed (>3 SD above the mean due to rat physically disturbing nozzle); water showed a significant interaction resembling food intake, where HCR drank significantly more than LCR but only by the 5th day of in the activity monitor.

As shown in Fig. 1B–E, physical activity variables (distance traveled; time spent in stereotypic movement, ambulatory movement, or resting; and horizontal, vertical, and ambulatory counts) were all significantly different between HCR and LCR, with HCR being more active; activity decreased after the first days of activity monitoring, with no significant interactions. Four- or five-day estrous cycles were detected in 10 HCR and 8 LCR (out of a total of 26 females). As seen in Fig. 1F, higher activity levels were seen on the 24-period prior to the day on which estrus was detected in HCR and LCR. There was no evidence of estrous cycle synchrony in this group of rats. Lastly, distance traveled showed a slight but significant decrease with age, and body weight increased with age; there were no significant interactions between age and line, however (HCR distance traveled went from 12.55 ± 1.23 to 11.35 ± 1.38 cm/min, and LCR went from 8.88 ± 0.76 to 7.98 ± 1.07 cm/min; HCR body weight went from 274.75 ± 10.90 g to 294.17 ± 23.50 g, and LCR went from 287.07 ± 4.83 g to 325.14 ± 7.45 g over the 6-month time period).

4.2. Genetically lean male rats showed elevated levels of daily physical activity

Male LCR weighed significantly more than male HCR (Fig. 2F). As illustrated in Fig. 2A, the ANOVA revealed that HCR ate significantly more than LCR over the 7-day measurement period, with no interaction and no significant change over time ($p = 0.065$). Though water intake reflected food intake over time, the ANOVA revealed no significant effects of group or day on water intake, and no interaction; mean 7-day water intake also did not differ between HCR and LCR (HCR, 23 ± 1 ml/day; LCR, 20 ± 1 ml/day; $p = 0.064$).

In general, HCR were more active than LCR, and all rats were more active in the first day they were housed in the activity monitor. All measures of physical activity except for time spent in stereotypic movement (including distance traveled; time spent resting or time spent ambulating; and horizontal, vertical, and ambulatory beam-break counts) showed significant interactions, as well as main effects of group (HCR > LCR) and day, with activity decreasing (and rest time increasing) after the first day in the activity monitor; these data are shown in Fig. 2B–E. For those variables showing a significant interaction, HCR showed a greater adaptation (i.e., change from day 1 to day 2) compared to LCR in time spent resting, distance traveled, and horizontal beam-break counts. For example, from day 1 to day 2, HCR decreased their distance traveled by 32%, whereas LCR decreased by 21%. Other activity variables showed trends ($0.01 < p < 0.05$) toward the same effect. Time spent in stereotypic movement showed significant main effects of day and group (HCR > LCR), with no interaction (Fig. 2D). Though not compared statistically (because they were measured separately), males appeared less active than females; on day 7 of activity monitoring, males covered 36% (HCR) and 37% (LCR) less distance than did females, and males rested 3% (HCR) and 4% (LCR) longer.

4.3. Lean female rats lost more weight and were more active during calorie restriction

Female HCR and LCR were subjected to 23 days of 50% CR with concurrent measurement of physical activity. HCR lost a significantly greater percentage of their baseline body weight (Fig. 3A). A similarly significant interaction was found whether or not body weight data from the ad lib feeding recovery days were included in the analysis, compared to the average 7-day baseline body weight prior to CR. At baseline, the LCR carried more fat and a higher percent body fat, whereas HCR had more lean mass and a higher percent lean mass. All rats lost both fat mass and lean mass during CR (Table 1); the proportion of weight lost through fat or lean mass did not differ between groups, though HCR females lost a greater proportion of their fat mass (76%, vs. 53% in LCR; $p < 0.05$), and HCR lost more lean mass (in grams), but not as a proportion of baseline lean mass (Table 1). Within group, there was no correlation between starting body weight and proportion of weight lost in either HCR ($r = -0.044$) or LCR ($r = 0.061$); starting body weights ranged 208–248 g in HCR, and 208–262 g in LCR. No correlation was seen between the proportion of body weight lost as lean mass as a function of baseline fat mass ($r = -0.082$; HCR, $r = -0.871$; LCR, $r = 0.723$) (see Ref. [44]).

Female HCR showed a significantly faster regain of lost weight, both as a percent of baseline BW (Fig. 3A) and in absolute mass (g); most of this was due to the weight regain on the first day of ad lib refeeding, when the HCR showed a significantly greater weight gain. The HCR reached LCR body weight by 1–3 days of refeeding, and reached similar percent of baseline body weights by 4 days. Long-term regain of body weight and composition did not significantly differ between groups, though there was a trend for the HCR to reach at least 99% of their baseline body weight faster than LCR (HCR: 9.25 ± 1.57 days; LCR: 11.13 ± 1.52 days). By 19 days after the end of CR, both groups were, on average, within 2 g of their baseline (pre-CR) fat mass and lean mass. Compared to LCR, HCR females ate more food before, during, and after CR; specifically, female HCR ate more food in the first 8 days of recovery from CR, as well as on recovery days 14 and 17.

In order to assess the role of physical activity in the enhanced CR-induced weight loss seen in HCR, we measured physical activity daily throughout CR. Consistent with previous findings [25–27], HCR were more active than LCR (Fig. 3B–F). In this group of rats, HCR and LCR did not overlap in their mean baseline distance traveled (range for HCR: 11.1–23.2 cm/min; LCR: 7.1–9.8 cm/min). Both female HCR and LCR showed a similar overall pattern of activity during CR where distance traveled showed a sharp increase during the first two days of CR (Fig. 3B), followed by a drop to or below baseline levels and a gradual suppression of physical activity throughout CR. The ANOVA comparing distance traveled in HCR and LCR over CR revealed a significant interaction where CR induced a greater suppression in distance traveled in HCR than in LCR. After the resumption of ad lib feeding, both groups of rats showed a transient 1-day elevation in physical activity to levels equivalent to each other, followed by a gradual return from CR levels

				Regain			
Lean mass	%Fat	%Lean	BW	Fat mass	Lean mass	%Fat	%Lean
150.95 ± 4.39*	**2.64** ± 1.07	**80.72** ± 0.37	**237.76** ± 5.04*	**21.05** ± 1.34	**180.03** ± 5.02	**8.89** ± 0.62	**75.68** ± 1.01
146.81 ± 4.08	**6.39** ± 0.93	**76.44** ± 0.90	**232.58** ± 5.70	**26.88** ± 1.45	**169.15** ± 4.67	**11.57** ± 0.62	**72.71** ± 0.60
0.25	**0.01**	<**0.001**	0.253	**0.005**	0.068	<**0.01**	<**0.05**
252.68 ± 5.75*	**9.26** ± 1.43*	**74.42** ± 1.43*	**410.65** ± 11.12	**58.82** ± 5.82	**293.65** ± 7.59*	**14.18** ± 1.24*	**71.62** ± 1.11
317.28 ± 22.23	**22.59** ± 0.98	**63.17** ± 0.88	**564.42** ± 41.95	**143.32** ± 15.63	**339.74** ± 21.41	**25.12** ± 1.06	**60.43** ± 1.00
0.002	<**0.001**	<**0.001**	<**0.001**	<**0.001**	**0.01**	<**0.001**	<**0.001**

to baseline activity levels over 12 days (Fig. 3B–F). Time spent resting was impacted differently in HCR and LCR; LCR rested more than HCR, but HCR gradually increased to LCR levels as CR proceeded (Fig. 3C). There was a steady recovery to baseline activity levels in the week after CR ended.

CR uncovered some differences between dimensions of physical activity. Time spent in stereotypic activity in HCR decreased to the level of LCR by the end of the 2nd week in CR (Fig. 3D). HCR stereotypic activity also did not show the characteristic elevation during the first 2 days of CR. This is in contrast to time spent ambulating, which was consistently longer in HCR than in LCR throughout CR, even with the gradual decrease in HCR ambulation during CR (Fig. 3E). Horizontal and ambulatory activity showed a similar pattern to distance traveled, where the HCR were more active than LCR at baseline; both groups showed a peak in activity at the onset of CR, followed by a steep decline then a gradual decline in activity during CR that was much more marked in HCR compared to LCR. HCR remained more active than LCR during CR, and both groups showed a peak in activity upon refeeding, followed by a gradual return to baseline levels in the 2nd week of refeeding that was more marked in HCR than in LCR. Vertical activity counts, on the other hand, followed a completely different pattern: HCR showed more vertical activity than did LCR before, during, and after CR, but showed no change over CR (Fig. 3F). In LCR, on the other hand, CR induced an increase in vertical activity, followed by a decrease after CR.

As a replication and to more closely examine the time course of weight change, a small group of HCR and LCR females (N = 4/line) were subjected to 50% CR for 5 weeks and body composition was measured weekly. Again, HCR showed a greater percent decrease in body weight (interaction, $p = 0.006$; Fig. 3G). Weight loss was due to a significant loss of both fat and lean mass, with HCR losing a significantly greater percentage of both fat and lean mass compared to LCR (interactions: $p < 0.01$; Fig. 3H–I). Total regain of fat and lean mass did not differ between HCR and LCR as each group reached their baseline levels one and two weeks (for fat mass and lean mass, respectively) after resumption of ad lib feeding.

4.4. Lean male rats lost more weight and were more active during calorie restriction

Calorie restriction induced significant weight loss in male rats over 21 days (Fig. 4A, Table 1). Compared to male LCR, male HCR showed significantly greater CR-induced weight loss as a proportion of their starting body weight (Fig. 4A); weight loss in grams also showed a significant interaction, with the much larger LCR losing more mass (Table 1). Within group, there was no significant correlation between starting body weight and proportion of weight lost in either HCR ($r = -0.327$) or LCR ($r = -0.243$), and ANCOVA detected a group difference (HCR > LCR) between proportion weight lost with starting body weight used as the covariate; starting body weights ranged 365–477 g in HCR, and 456–769 g in LCR. Starting fat mass was significantly negatively correlated with the proportion of weight lost as lean mass overall ($r = -0.871$; HCR, $r = -0.535$; LCR, $r = -0.412$). Baseline food intake, and therefore 50% CR food intake, was higher in HCR than in LCR. Food intake during recovery from CR showed a significant interaction and main effect of time, but no main effect of group; HCR and LCR males did not differ in their food intake in the first 8 days of recovery, but on days 9–14, LCR ate significantly more than did HCR males. Water intake reflected food intake, decreasing during CR, but with no difference between HCR and LCR.

Over the course of 21 days of 50% CR, male rats lost fat mass along with lean mass, but the change in body composition differed between groups. HCR lost 25.07 g (±2.34 g) of fat, and the LCR lost 57.65 g (±6.00 g) fat, which was significantly more; thus there were significant main effects of CR and group, with a significant interaction in that LCR lost more grams of fat. The same effects were seen in percent body fat between groups. Lean mass was also lost during CR in all rats; there were significant main effects of CR and group, with a significant interaction where HCR lost more grams of lean mass (43.20 g ± 2.76 g) compared to LCR (25.04 g ± 2.77 g). HCR lost a greater percentage of their baseline body fat (47% ± 5%) and their baseline lean mass (15% ± 1%) compared to LCR (which lost 34% ± 2% of their fat mass, and 8% ± 1% of their lean mass). As shown in Fig. 4I, whereas LCR males lost most of their body weight in fat, and less in lean mass, HCR lost most of their body weight as lean mass rather than fat mass, indicating that HCR were less able to conserve lean mass during CR. (The remaining grams of body weight lost beyond fat and lean mass was composed of bones, skin, fur, and free water; this did not differ between groups).

As shown in Fig. 4B–H, most physical activity variables (distance traveled, stereotypic time, ambulatory time, horizontal counts, ambulatory counts) showed similar patterns over CR. The ability of CR to suppress activity was seen almost exclusively in the HCR (significant interaction, and main effects of line and time, compared to the baseline of mean activity for 5 days before the onset of CR). HCR were significantly more active than LCR (main effect of line) in every dimension of activity except for ambulatory activity counts (trend of $p = 0.076$); time spent resting showed the opposite effect (LCR > HCR). As seen in the females, physical activity increased in the first 1–3 days of CR in both HCR and LCR (Fig. 4); this was followed by a steep decrease in activity (over days 4–6 of CR) in HCR, then a gradual decline in activity in all rats over the remaining time in CR. Over time in CR, HCR decreased their activity to reach levels similar to (i.e., not statistically significantly different from) LCR, but the timing of this differed across dimension of physical activity: by days 8–11 in stereotypic time, horizontal counts, and ambulatory counts; by day 13 for rest time; by day 16 for distance traveled; and HCR > LCR through all but the last day for ambulatory time (see Figure B–H). CR significantly suppressed several dimensions of activity in HCR, but not LCR (distance traveled; vertical, horizontal, and ambulatory counts, as well as the opposing change in time spent resting). In LCR, CR significantly suppressed only time spent ambulating (on days 14 and 15) and time in stereotypic movement (on day 14).

Resumption of ad lib feeding resulted in regain of lost weight, and this regain was significantly faster in HCR males than in LCR males (significant interaction between groups in weight regain). HCR regained significantly more mass (g) than LCR in 3 out of the first 4 days of ad lib feeding. By the 14th day of ad lib feeding, HCR had reached

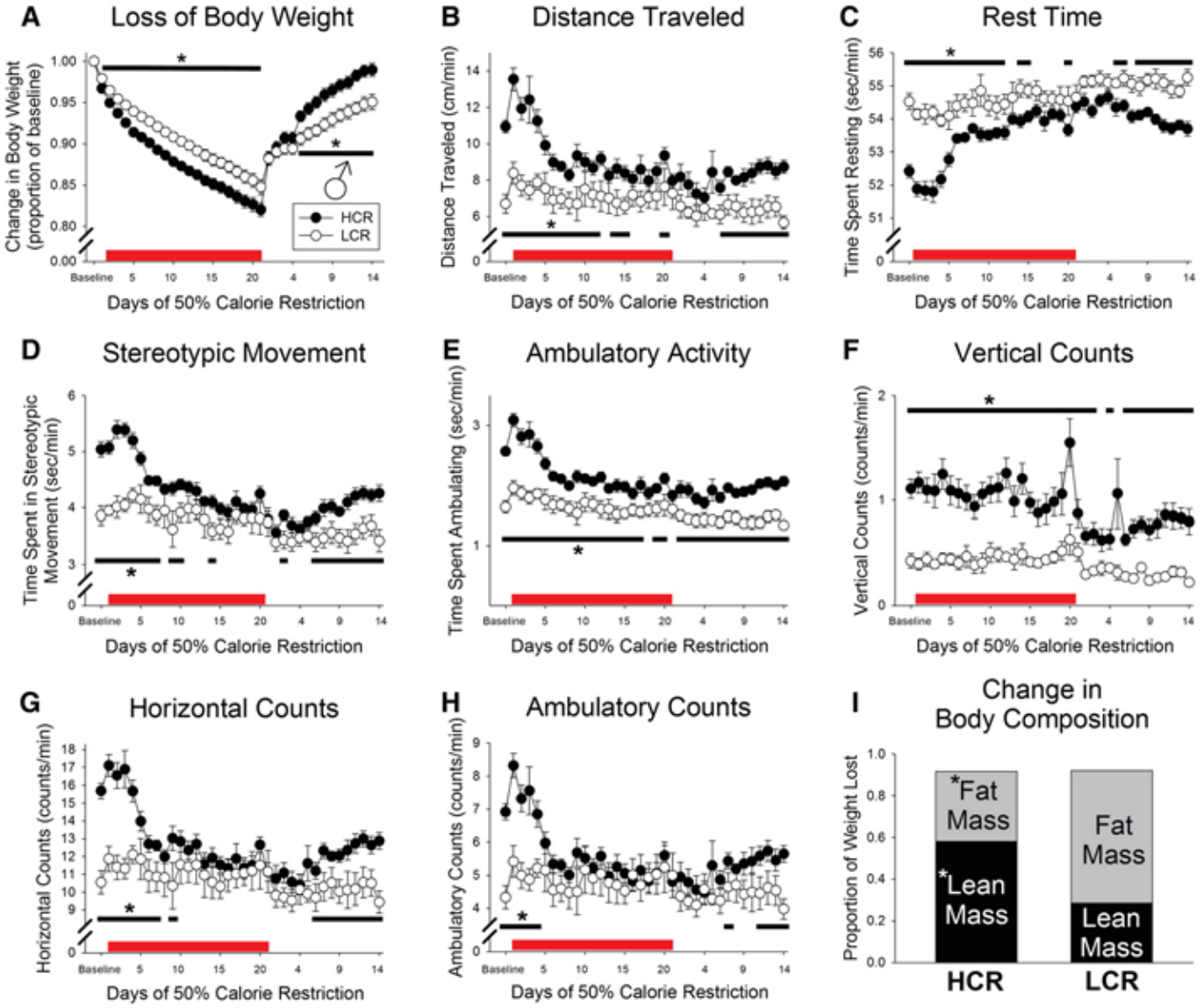

Fig. 4. 50% calorie restriction suppressed physical activity and produced greater weight loss in more physically active male rats selected for high aerobic capacity (HCR) compared to low-capacity (LCR) males (CR; 21-day duration represented by line adjacent to X-axis). (A) HCR males lost significantly more weight than did male LCR as a proportion of their baseline body weight. CR induced a characteristic change in physical activity levels in both groups of male rats where activity levels were elevated on the first 1–3 days of CR, then suppressed over the course of CR only in HCR, with a gradual rebound after resumption of ad-lib food intake. CR differentially suppressed distinct dimensions of physicals activity. In male HCR, stereotypic movement (D), as well as horizontal (G) and ambulatory (H) activity counts (beam-break counts/min), were suppressed to the levels of male LCR during the last half of CR. Distance traveled (B) remained higher in HCR males through most of CR, until the final week; time spent sedentary (C) showed the inverse pattern. (E) Male HCR spent significantly more time in ambulatory activity on nearly every day of CR. (F) HCR males showed significantly more vertical activity counts than did LCR throughout the measurement period, though the impact of CR on vertical activity was noticeably different than CR effects on all other dimensions of activity (B–E, G–H), which gradually recovered toward baseline levels in the two weeks after resumption of ad-lib feeding though the recovery was less robust in LCR. This paralleled the post-CR weight regain (A), which was faster and more pronounced in HCR than in LCR males. (I) Whereas most of the weight LCR males lost was due to loss of fat mass, HCR males lost more weight through loss of lean mass. *$p < 0.05$, HCR ≠ LCR on days indicated by horizontal line.

99 ± 0.01% of their pre-CR body weight, whereas LCR regained 95 ± 0.00% of their baseline weight by this time. This was mainly attributable to fat regain; after 14 days of ad-lib feeding, HCR males had regained 103% (±2%) of their fat mass, but LCR males had regained only 83% (±2%) of their fat mass ($p < 0.001$); both groups had regained over 99% of their lean mass by this time. Physical activity remained suppressed for several days after resumption of ad lib feeding, and showed a significant interaction between HCR and LCR over the course of refeeding in some dimensions (rest time and stereotypic time; horizontal counts), but not others (distance traveled, ambulatory time, ambulatory counts, vertical counts), where HCR showed gradually increasing activity levels, and LCR did not. Whereas most dimensions of activity during recovery remained significantly suppressed relative to baseline levels in both HCR and LCR males (except ambulatory counts, which were significantly below baseline levels only in HCR; Fig. 4E), the magnitude of difference from baseline was much greater in HCR than in LCR, despite the greater increase in activity levels in HCR over the 2 weeks of recovery from CR.

5. Discussion

High levels of daily "spontaneous" physical activity are consistently coupled with leanness and healthy metabolic outcomes [25–31,45]. Here, we demonstrate that highly active rats show enhanced weight loss during food restriction, supporting the assertion that physical activity opposes metabolic thriftiness during food restriction. Obesity-prone LCR lost less weight than HCR and, in females, conserved adiposity. Moreover, the highly physically active HCR maintained higher activity

levels than LCR throughout most or all of the duration of calorie restriction. This is concordant with the heightened levels of baseline physical activity energy expenditure (i.e., NEAT) seen in HCR [25]. Taken together, this implicates elevated EE, especially NEAT from elevated daily physical activity, in the ability to lose weight during food restriction.

It is accepted that obese people and obese laboratory animals are less active than lean ones (reviewed in Refs. [4,6,7]). Arguments have been made that this dichotomy is intrinsic to the phenotype or, alternatively, that it is secondary to the development of obesity. We observe reliably elevated long-term physical activity in HCR relative to LCR, regardless of sex and the accompanying differences in body weight (Figs. 1–2) [25–27], demonstrating that elevated physical activity is associated with the lean phenotype rather than with body size or body fat of the animal. Furthermore, it is clear that the LCR are physically capable of being more active [46], as evidenced by the elevated activity levels upon resumption of ad lib feeding seen in females LCR (Fig. 3B). Though activity levels rise upon introduction to a new environment in all rats, and vary day-to-day, the lean HCR are consistently more active than obesity-prone LCR on nearly any given day (Figs. 1–2). This, combined with a remarkably low within-group variance, demonstrates that the phenotypic difference in physical activity is consistent, robust, and not secondary to a hypothetical burden or disability experienced by the obesity-prone LCR. Conditions of activity measurement, housing or environment, and duration of monitoring all influence experimental outcomes [47,48]. Even in the HCR and LCR, which show robust and easily detectable differences in daily activity under a variety of conditions (e.g., cage sizes, temperatures) [25–27], short-term (e.g., 2-to-3-h) activity does not differ [26,49], underscoring the importance of 24-h measurement durations after sufficient acclimation when assessing daily physical activity levels [47].

Here, we establish that this elevated physical activity seen in HCR is consistent across sex, age, and body size over long-term measurement (Figs. 1 & 2). More significantly, we demonstrate for the first time that the high-activity rats lose significantly more weight than their low-activity counterparts when subjected to equivalent food restriction (Figs. 3 & 4). On average in males, LCR are considerably larger than HCR (here, +178 g at baseline; Table 1), thus male LCR lose more weight in absolute terms compared to male HCR (90 g in LCR vs. 74 g in HCR; Table 1). This translates to a significantly greater weight loss as a proportion of baseline weight in male HCR (18%, compared to 15% in male LCR; Fig. 4); this difference persisted even after baseline body weight was accounted for, and baseline body weight was not a significant predictor of proportional weight loss within any group. Levels of baseline adiposity in males may partly account for differences in weight loss, especially the contribution of lean mass to overall weight loss (Fig. 4I) [44]; for example, male LCR were particularly adept at retaining their lean mass. In contrast to male rats, the use of female rats presents an experimental advantage in minimizing group differences in body weight and adiposity, allowing for a more direct and straightforward group comparison. Female HCR and LCR began at similar mean body weights (Table 1); nonetheless, female HCR lost more weight than female LCR both as a percentage of their body weight (19% in HCR, 16% in LCR; Fig. 2) and in absolute mass (45 g in HCR; 37.5 g in LCR; Table 1). The greater weight loss in HCR was not secondary to baseline adiposity, and female HCR and LCR did not differ in the proportion of the weight lost as fat vs. lean mass. Interestingly, female HCR showed similar proportional weight loss as their male counterparts, as did female and male LCR, despite the females' much smaller overall baseline body weights in both groups, arguing against the notion that HCRs' greater proportional weight loss was solely a consequence of their lower baseline mass or adiposity. Similarly, enhanced conservation of body mass has been documented in a genetic model of obesity in rats, which also shows improved survival and resistance to starvation [38,39]. Altogether, these data emphasize the contrasting phenotypic response to energy restriction independent of baseline body weight, and point to intrinsic aerobic capacity and daily physical activity as key predictors of weight loss.

Food restriction has predictable effects on physical activity in multiple vertebrate classes and species [48,50–52], and the rats examined here followed the predicted pattern (Figs. 3 & 4). Reduced food availability stimulated increased physical activity levels in the first 1–3 days of exposure, sometimes attributed to a foraging response [50,51,53], followed by a slow decline in activity levels and a long-term suppression below baseline activity levels. Along with losing more weight, HCR showed consistently high levels of physical activity not only before but even during the 3 weeks of CR (Figs. 3 & 4), supporting the hypothesis that reduced physical activity levels during food shortage may serve to conserve energy and therefore body mass. Measurement of energy expenditure during CR, specifically the energy expenditure of physical activity, is needed to test this part of the hypothesis, however. Apart from a transient, moderate increase in activity in female rats upon refeeding (Fig. 3B, E), activity levels increased gradually, mirroring the reestablishment of baseline body weights, with groups of rats that re-gained weight more rapidly (females compared to males; male HCR compared to male LCR) also restoring their activity levels to baseline levels earlier (Figs. 3, 4). Just as physical activity diminished over the course of weight loss, the recovery of daily activity levels lagged behind weight regain. These findings have implications for those attempting to maintain activity levels after diet-induced weight loss. Indeed, the marked suppression of activity levels would counteract efforts to maintain weight loss, even in individuals not prone to weight gain [35,36,54–57].

Food restriction unmasked differences between the dimensions of physical activity. Whereas most activity measures decreased during CR, vertical activity did not significantly decrease in any group, and actually was elevated in males during CR relative to recovery (Fig. 4F) as well as in female LCR during CR relative to either before or after CR (Fig. 3F). It is likely that vertical activity (e.g., rearing) may be a distinct aspect of physical activity, potentially under different regulatory control [47], and linked to high environmental reactivity [58]. Similarly, prolonged CR also impacted stereotypic activity (e.g., grooming and other repetitive movements; Figs. 3D, 4D) more than ambulatory movements (e.g., walking about the chamber; Figs. 3E, 4E); it is possible that this change in stereotypic activity results from decreases in meal frequency and related postprandial grooming. In both males and females, stereotypic activity in HCR was decreased to the level of LCR during CR, whereas HCR time spent in ambulatory movement remained elevated over LCR for much of CR. Interestingly, the sustained increase in time spent ambulating in HCR was not reflected in ambulatory activity counts in males (Fig. 4H; ambulatory counts in females, HCR > LCR in all by 5 days during CR), implicating a difference in ambulatory speed.

Regardless of sex, HCR were more active than LCR. Females showed more inter-individual and day-to-day variance, however, likely attributable to their estrous cycle (Fig. 1F). These cyclical variations in activity are consistent with the general pattern of wheel running described over the estrous cycle [48], with higher activity levels preceding estrus, but with a much lower amplitude. Given the relatively minor increase in physical activity near estrus, it is unlikely that estrous cycles would interfere with the detection of differences in activity between HCR and LCR, or account for the differences we document on any single day. Female rats also showed a slight age-related decline in physical activity. The design of this study did not allow for direct statistical comparison of activity levels between male and female rats, but descriptive observations are consistent with reports that females are more active than males (reviewed in Ref. [59]). Considering the substantial difference in body sizes between males and females, any differences observed between males and females were relatively minor compared to the overall associations between phenotype, physical activity, and weight loss seen in both sexes.

These data also speak to modern interpretations of the thrifty genotype hypothesis [34,38,39,60–62]. The lower proportional weight loss in the obesity-prone LCR (Figs. 3A, 4A) is consistent with the idea that the

tendency to become obese is accompanied by a relative energetic thriftiness and a resistance to the deleterious effects of famine. The hypothesis that obesity-prone genotypes confer a fitness advantage in food-restricted environments is also supported by results showing that monogenically obese rats not only defend body mass during energy restriction but also resist starvation [39]. While baseline body weight and weight loss in HCR/LCR rats fit this hypothesis, the finding that HCR regain weight during refeeding does not. Alternatives to the thrifty genotype hypothesis propose that obesity arises after a release from predation pressures removes selection against weight gain. Similarly, the "dual intervention point" hypothesis [62–64] suggests upper and lower limits for the regulation of body mass set by the opposing pressures of predation and starvation, respectively. Altogether, behavioral and physiological findings from high- and low-capacity runners support the hypothesis that selection based on different environmental pressures (for example, predation pressure or thermogenic capacity vs. starvation) experienced in different contexts can yield populations that differ in obesity propensity [61]. Moreover, the trait selected upon (here, aerobic capacity) does not need to be body weight or adiposity to result in differences in body weight, body composition, and thriftiness. In light of these hypotheses, our data suggest that there may be a trade-off between thriftiness (i.e., resistance to starvation) and aerobic capacity that depends on the selective pressures at play for a particular population, such as predation or the reliability of food availability. HCR would be predicted to be more resistant to predation due to their running endurance and hypervigilant behavioral profile [65], regardless of their body size or adiposity, yet lack thrifty defense of body weight during calorie restriction, as demonstrated here. On the other hand, LCR would be more resistant to food shortage, yet more vulnerable to predation. Consistent with this, populations on islands more distantly separated from predators show a "tame" behavioral profile similar to LCR [66]. It is possible that two traits which show large inter-individual variance in a normal population—intrinsic aerobic capacity and energetic thriftiness—are not mechanistically compatible because metabolic thriftiness depends on low aerobic capacity. The proposal that selection advantages are conferred by adaptations in thermoregulatory control [61] is especially germane in the light of data demonstrating overlap in the molecular mechanisms underlying thermoregulation, aerobic capacity, and energetic cost of activity [67–69].

6. Conclusions

Here we show for the first time that low intrinsic fitness is tightly coupled to metabolic thriftiness and thus to defense of body weight, and that low or suppressed physical activity may aid in energy conservation. Taken together with previous reports [25–27,49], these data strongly support the hypothesis that there is an intrinsically lean phenotype associated with high intrinsic aerobic capacity, high daily activity levels, and accelerated weight loss during food restriction. This elevated physical activity is seen regardless of sex or body size. Related to human health, similar links have been observed between aerobic capacity, physical activity, leanness, and health in people [2,27,31,45,70]. The calories used in this elevated physical activity, including during calorie restriction, could serve to enhance energy expenditure and contribute to the relative lack of energetic thriftiness.

Competing interests

None of the authors have any conflicts of interest to disclose.

Author contributions

The studies were performed in the laboratory of CMN. Conception and design of experiments: MES, CMN, SLB, and LGK. Collection, analysis and interpretation of data: MES, CMN, and KZB. Drafting of the article or revising it critically for important intellectual content: all authors.

All authors have approved the final version of the manuscript, all authors qualified for authorship, and all those who qualify for authorship are listed.

Funding

This work is supported by NIH R01NS055859, NIH R15DK097644, and AHA GIA 410805 to CMN. The LCR–HCR rat model system was supported by the Office of Research Infrastructure Programs/OD grant R24OD010950 and by grant R01DK099034 (to LGK and SLB) from the National Institutes of Health. SLB was also supported by National Institutes of Health grants R01DK077200 and R01GM104194.

Acknowledgments

We acknowledge the generous assistance from Mark Moser and his staff in the KSU Department of Biological Sciences vivarium, and the expert care of the rat colony provided at the University of Michigan by Molly Kalahar and Lori Heckenkamp. Contact LGK (lgkoch@med.umich.edu) or SLB (brittons@umich.edu) for information on the LCR and HCR rats: these rat models are maintained as an international resource with support from the Department of Anesthesiology at the University of Michigan, Ann Arbor, Michigan.

References

[1] Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011–2012. JAMA 2014;311:806–14.
[2] Levine JA, Eberhardt NL, Jensen MD. Role of nonexercise activity thermogenesis in resistance to fat gain in humans. Science 1999;283:212–4.
[3] Snitker S, Tataranni PA, Ravussin E. Spontaneous physical activity in a respiratory chamber is correlated to habitual physical activity. Int J Obes Relat Metab Disord 2001;25:1481–6.
[4] Thorburn AW, Proietto J. Biological determinants of spontaneous physical activity. Obes Rev 2000;1:87–94.
[5] Mustelin L, Silventoinen K, Pietilainen K, Rissanen A, Kaprio J. Physical activity reduces the influence of genetic effects on BMI and waist circumference: a study in young adult twins. Int J Obes (Lond) 2009;33:29–36.
[6] Kotz CM, Teske JA, Billington CJ. Neuroregulation of nonexercise activity thermogenesis and obesity resistance. Am J Physiol Regul Integr Comp Physiol 2008;294:R699–710.
[7] Novak CM, Levine JA. Central neural and endocrine mechanisms of non-exercise activity thermogenesis and their potential impact on obesity. J Neuroendocrinol 2007;19:923–40.
[8] University of Minnesota, Laboratory of Physiological Hygiene, Keys AB. The biology of human starvation. Minneapolis: University of Minnesota Press; 1950.
[9] de Groot LC, van Es AJ, van Raaij JM, Vogt JE, Hautvast JG. Adaptation of energy metabolism of overweight women to alternating and continuous low energy intake. Am J Clin Nutr 1989;50:1314–23.
[10] Martin CK, Heilbronn LK, de Jonge L, DeLany JP, Volaufova J, Anton SD, et al. Effect of calorie restriction on resting metabolic rate and spontaneous physical activity. Obesity (Silver Spring) 2007;15:2964–73.
[11] Weed JL, Lane MA, Roth GS, Speer DL, Ingram DK. Activity measures in rhesus monkeys on long-term calorie restriction. Physiol Behav 1997;62:97–103.
[12] den Hoed M, Westerterp KR. Body composition is associated with physical activity in daily life as measured using a triaxial accelerometer in both men and women. Int J Obes (Lond) 2008;32:1264–70.
[13] Ruderman NB, Schneider SH, Berchtold P. The "metabolically-obese," normal-weight individual. Am J Clin Nutr 1981;34:1617–21.
[14] Klem ML, Wing RR, McGuire MT, Segale HM, Hill JO. A descriptive study of individuals successful at long-term maintenance of substantial weight loss. Am J Clin Nutr 1997;66:239–46.
[15] Hankinsona AL, Daviglus ML, Bouchard C, Carnethon M, Lewis CE, Schreiner PJ, et al. Maintaining a high physical activity level over 20 years and weight gain. JAMA 2010;304:2603–10.
[16] Pietilainen KH, Kaprio J, Borg P, Plasqui G, Yki-Jarvinen H, Kujala UM, et al. Physical inactivity and obesity: a vicious circle. Obesity (Silver Spring) 2008;16:409–14.
[17] Tucker JM, Tucker LA, Lecheminant J, Bailey B. Obesity increases risk of declining physical activity over time in women: a prospective cohort study. Obesity (Silver Spring) 2013;21:E715–20.
[18] Lakka TA, Bouchard C. Physical activity, obesity and cardiovascular diseases. Handb Exp Pharmacol 2005;137–63.
[19] Ladabaum U, Mannalithara A, Myer PA, Singh G. Obesity, abdominal obesity, physical activity, and caloric intake in U.S. adults: 1988–2010. Am J Med 2014;127:717–27.
[20] Ravussin E, Bogardus C. Energy balance and weight regulation: genetics versus environment. Br J Nutr 2000;83(Suppl. 1):S17–20.

[21] Zurlo F, Ferraro RT, Fontvielle AM, Rising R, Bogardus C, Ravussin E. Spontaneous physical activity and obesity: cross-sectional and longitudinal studies in Pima Indians. Am J Physiol 1992;263:E296–300.
[22] Joosen AM, Gielen M, Vlietinck R, Westerterp KR. Genetic analysis of physical activity in twins. Am J Clin Nutr 2005;82:1253–9.
[23] Whitaker KL, Jarvis MJ, Boniface D, Wardle J. The intergenerational transmission of thinness. Arch Pediatr Adolesc Med 2011;165:900–5.
[24] Westerterp KR. Physical activity as determinant of daily energy expenditure. Physiol Behav 2008;93:1039–43.
[25] Gavini CK, Mukherjee S, Shukla C, Britton SL, Koch LG, Shi H, et al. Leanness and heightened nonresting energy expenditure: role of skeletal muscle activity thermogenesis. Am J Physiol Endocrinol Metab 2014;306:E635–47.
[26] Novak CM, Escande C, Burghardt PR, Zhang M, Barbosa MT, Chini EN, et al. Spontaneous activity, economy of activity, and resistance to diet-induced obesity in rats bred for high intrinsic aerobic capacity. Horm Behav 2010;58:355–67.
[27] Novak CM, Escande C, Gerber SM, Chini EN, Zhang M, Britton SL, et al. Endurance capacity, not body size, determines physical activity levels: role of skeletal muscle PEPCK. PLoS One 2009;4:e5869.
[28] McGuire KA, Ross R. Incidental physical activity is positively associated with cardiorespiratory fitness. Med Sci Sports Exerc 2011;43:2189–94.
[29] Aspenes ST, Nauman J, Nilsen TI, Vatten L, Wisloff U. Physical activity as a long term predictor of peak oxygen uptake: the HUNT-study. Med Sci Sports Exerc 2011;43:1675–9.
[30] Aspenes ST, Nilsen TI, Skaug EA, Bertheussen GF, Ellingsen O, Vatten L, et al. Peak oxygen uptake and cardiovascular risk factors in 4,631 healthy women and men. Med Sci Sports Exerc 2011;43:1465–73.
[31] Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, et al. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. JAMA 2009;301:2024–35.
[32] Koch LG, Britton SL, Wisloff U. A rat model system to study complex disease risks, fitness, aging, and longevity. Trends Cardiovasc Med 2012;22:29–34.
[33] Wisloff U, Najjar SM, Ellingsen O, Haram PM, Swoap S, Al-Share Q, et al. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. Science 2005;307:418–20.
[34] Prentice AM, Hennig BJ, Fulford AJ. Evolutionary origins of the obesity epidemic: natural selection of thrifty genes or genetic drift following predation release? Int J Obes (Lond) 2008;32:1607–10.
[35] Redman LM, Heilbronn LK, Martin CK, de Jonge L, Williamson DA, Delany JP, et al. Metabolic and behavioral compensations in response to caloric restriction: implications for the maintenance of weight loss. PLoS One 2009;4:e4377.
[36] Wadden TA, Neiberg RH, Wing RR, Clark JM, Delahanty LM, Hill JO, et al. Four-year weight losses in the Look AHEAD study: factors associated with long-term success. Obesity (Silver Spring) 2011;19:1987–98.
[37] Vaanholt LM, Magee V, Speakman JR. Factors predicting individual variability in diet-induced weight loss in MF1 mice. Obesity (Silver Spring) 2012;20:285–94.
[38] Diane A, Pierce WD, Heth CD, Russell JC, Richard D, Proctor SD. Feeding history and obese-prone genotype increase survival of rats exposed to a challenge of food restriction and wheel running. Obesity (Silver Spring) 2012;20:1787–95.
[39] Pierce WD, Diane A, Heth CD, Russell JC, Proctor SD. Evolution and obesity: resistance of obese-prone rats to a challenge of food restriction and wheel running. Int J Obes (Lond) 2010;34:589–92.
[40] Gardner CD, Kim S, Bersamin A, Dopler-Nelson M, Otten J, Oelrich B, et al. Micronutrient quality of weight-loss diets that focus on macronutrients: results from the A TO Z study. Am J Clin Nutr 2010;92:304–12.
[41] Nutrient requirements of laboratory animals: fourth revised edition. Washington, DC: The National Academies Press; 1995.
[42] Bowman TA, Ramakrishnan SK, Kaw M, Lee SJ, Patel PR, Golla VK, et al. Caloric restriction reverses hepatic insulin resistance and steatosis in rats with low aerobic capacity. Endocrinology 2010;151:5157–64.
[43] Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: some helpful considerations. Braz J Biol 2002;62:609–14.
[44] Forbes GB. Body fat content influences the body composition response to nutrition and exercise. Ann N Y Acad Sci 2000;904:359–65.
[45] 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.
[46] Haram PM, Kemi OJ, Lee SJ, Bendheim MO, Al-Share QY, Waldum HL, et al. Aerobic interval training vs. continuous moderate exercise in the metabolic syndrome of rats artificially selected for low aerobic capacity. Cardiovasc Res 2009;81:723–32.
[47] Teske JA, Perez-Leighton CE, Billington CJ, Kotz CM. Methodological considerations for measuring spontaneous physical activity in rodents. Am J Physiol Regul Integr Comp Physiol 2014;306:R714.
[48] Novak CM, Burghardt PR, Levine JA. The use of a running wheel to measure activity in rodents: relationship to energy balance, general activity, and reward. Neurosci Biobehav Rev 2012;36:1001–14.
[49] Shukla C, Britton SL, Koch LG, Novak CM. Region-specific differences in brain melanocortin receptors in rats of the lean phenotype. Neuroreport 2012;23:596–600.
[50] Novak CM, Jiang X, Wang C, Teske JA, Kotz CM, Levine JA. Caloric restriction and physical activity in zebrafish (*Danio rerio*). Neurosci Lett 2005;383:99–104.
[51] Lynn SE, Breuner CW, Wingfield JC. Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird. Horm Behav 2003;43:150–7.
[52] Severinsen T, Munch IC. Body core temperature during food restriction in rats. Acta Physiol Scand 1999;165:299–305.
[53] Williams TD, Chambers JB, Henderson RP, Rashotte ME, Overton JM. Cardiovascular responses to caloric restriction and thermoneutrality in C57BL/6J mice. Am J Physiol Regul Integr Comp Physiol 2002;282:R1459–67.
[54] van Baak MA, van Mil E, Astrup AV, Finer N, Van Gaal LF, Hilsted J, et al. Leisure-time activity is an important determinant of long-term weight maintenance after weight loss in the Sibutramine Trial on Obesity Reduction and Maintenance (STORM trial). Am J Clin Nutr 2003;78:209–14.
[55] Vogels N, Egger G, Plasqui G, Westerterp KR. Estimating changes in daily physical activity levels over time: implication for health interventions from a novel approach. Int J Sports Med 2004;25:607–10.
[56] Wadden TA, West DS, Neiberg RH, Wing RR, Ryan DH, Johnson KC, et al. One-year weight losses in the Look AHEAD study: factors associated with success. Obesity (Silver Spring) 2009;17:713–22.
[57] Wang X, Lyles MF, You T, Berry MJ, Rejeski WJ, Nicklas BJ. Weight regain is related to decreases in physical activity during weight loss. Med Sci Sports Exerc 2008;40:1781–8.
[58] Thiel CM, Muller CP, Huston JP, Schwarting RK. High versus low reactivity to a novel environment: behavioural, pharmacological and neurochemical assessments. Neuroscience 1999;93:243–51.
[59] Tou JC, Wade CE. Determinants affecting physical activity levels in animal models. Exp Biol Med (Maywood) 2002;227:587–600.
[60] Speakman JR. Thrifty genes for obesity, an attractive but flawed idea, and an alternative perspective: the 'drifty gene' hypothesis. Int J Obes (Lond) 2008;32:1611–7.
[61] Sellayah D, Cagampang FR, Cox RD. On the evolutionary origins of obesity: a new hypothesis. Endocrinology 2014;155:1573–88.
[62] Speakman JR. A nonadaptive scenario explaining the genetic predisposition to obesity: the "predation release" hypothesis. Cell Metab 2007;6:5–12.
[63] Levitsky DA. Putting behavior back into feeding behavior: a tribute to George Collier. Appetite 2002;38:143–8.
[64] Zub K, Szafranska PA, Konarzewski M, Speakman JR. Effect of energetic constraints on distribution and winter survival of weasel males. J Anim Ecol 2011;80:259–69.
[65] Burghardt PR, Flagel SB, Burghardt KJ, Britton SL, Gerard-Koch L, Watson SJ, et al. Risk-assessment and coping strategies segregate with divergent intrinsic aerobic capacity in rats. Neuropsychopharmacology 2011;36:390–401.
[66] Cooper Jr WE, Pyron RA, Garland Jr T. Island tameness: living on islands reduces flight initiation distance. Proc Biol Sci 2014;281:20133019.
[67] Schaeffer PJ, Villarin JJ, Pierotti DJ, Kelly DP, Lindstedt SL. Cost of transport is increased after cold exposure in *Monodelphis domestica*: training for inefficiency. J Exp Biol 2005;208:3159–67.
[68] Schaeffer PJ, Villarin JJ, Lindstedt SL. Chronic cold exposure increases skeletal muscle oxidative structure and function in *Monodelphis domestica*, a marsupial lacking brown adipose tissue. Physiol Biochem Zool 2003;76:877–87.
[69] Villarin JJ, Schaeffer PJ, Markle RA, Lindstedt SL. Chronic cold exposure increases liver oxidative capacity in the marsupial *Monodelphis domestica*. Comp Biochem Physiol A Mol Integr Physiol 2003;136:621–30.
[70] Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE. Exercise capacity and mortality among men referred for exercise testing. N Engl J Med 2002;346:793–801.