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RESEARCH ARTICLE

Obesity, Diabetes and Energy Homeostasis

High-fat feeding disrupts daily eating behavior rhythms in obesity-prone but not in obesity-resistant male inbred mouse strains

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Abstract

Abnormal meal timing, like skipping breakfast and late-night snacking, is associated with obesity in humans. Disruption of daily eating rhythms also contributes to obesity in mice. When fed a high-fat diet, male C57BL/6J mice have disrupted eating behavior rhythms and they become obese. In contrast to obesity-prone C57BL/6J mice, some inbred strains of mice are resistant to high-fat diet-induced obesity. In this study, we sought to determine whether there are distinct effects of high-fat feeding on daily eating behavior rhythms in obesity-prone and obesity-resistant male mice. Male obesity-prone (C57BL/6J and 129X1/SvJ) and obesity-resistant (SWR/J and BALB/cJ) mice were fed low-fat diet or high-fat diet for 6 wk. Consistent with previous studies, obesity-prone male mice gained more weight and adiposity during high-fat diet feeding than obesity-resistant male mice. The amplitude of the daily rhythm of eating behavior was markedly attenuated in male obesity-prone mice fed high-fat diet, but not in obesity-resistant males. In contrast, high-fat feeding did not differentially affect locomotor activity rhythms in obesity-prone and obesity-resistant male mice. Together, these data suggest that regulation of the daily rhythm of eating may underlie the propensity to develop diet-induced obesity in male mice.

circadian; eating behavior rhythm; high-fat diet; mouse; obesity

INTRODUCTION

Aberrant meal timing increases the risk for obesity and cardiometabolic diseases (1). Eating at the wrong time of day, by skipping breakfast or eating late at night, is associated with obesity, metabolic syndrome, and increased risk for coronary heart disease (1–13). The circadian system coordinates daily, ~24-h rhythms of behavior (such as eating and locomotion) and physiology (such as glucose metabolism) with environmental cycles (14). It is hypothesized that disruption of this fine tuning of internal rhythms with external cycles, which occurs during aberrant meal timing, contributes to poor health.

Disruption of the daily eating rhythm also contributes to obesity in C57BL/6J male mice. Male C57BL/6J mice are obesity prone, so they readily gain weight when fed a high-fat diet (15). The daily rhythm of eating behavior is also markedly altered during high-fat feeding. When male C57BL/6J mice are fed a low-fat diet, they have high-amplitude rhythmic eating behavior that is consolidated during the night (16–18). However, when they are fed a high-fat diet, their eating behavior rhythm becomes low amplitude or arrhythmic (16–18). Moreover, diet-induced obesity is inhibited in male C57BL/6J mice when they are fed a high-fat diet only during the active phase (nighttime), demonstrating that meal timing is a determinant of obesity in these mice (19–21).

It is well established that some inbred strains of mice, such as C57BL/6J and 129X1/SvJ mice, are obesity prone, while others, such as SWR/J and BALB/cJ, are resistant to diet-induced obesity (15, 22–26). Previous studies have shown that diet-induced weight gain in obesity-prone strains is not simply attributed to greater caloric intake or lower spontaneous activity levels compared with obesity-resistant strains (23, 25–29). Since the timing of food consumption is a critical factor in developing diet-induced obesity in C57BL/6J male mice (19, 21), we hypothesized that obesity-resistant strains would maintain robust daily rhythms of eating behavior when fed a high-fat diet. If so, then this could be a mechanism underlying their resistance to obesity. Herein we investigated the effects of high-fat diet consumption on

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daily rhythms of eating behavior and locomotor activity in obesity-prone C57BL/6J and 129X1/SvJ male mice and in obesity-resistant SWR/J and BALB/cJ male mice.

METHODS

Animals and Experimental Protocol

C57BL/6J (stock no. 000664), 129X1/SvJ (stock no. 000691), SWR/J (stock no. 000689), and BALB/cJ (stock no. 000651) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and used as breeders to generate mice for experiments. All mice for experiments were bred in our animal facility in a 12-h:12-h light-dark cycle, and breeders and pups were fed standard rodent diet (Teklad 2918 Irradiated: 18% kcal fat). At 21 days old, pups were weaned and group housed with same-sex siblings in 12-h:12-h light-dark with standard rodent diet and water ad libitum. At 7 wk old, male mice were singly housed in cages (33 × 17 × 14 cm) with locked wheels (wheels were present but could not rotate) in 12-h:12-h light-dark (white LEDs, 250–350 lux) and fed low-fat diet ad libitum (10% kcal fat, Research Diets D12450K; see Supplemental Table S1; all Supplemental materials are available at https://doi.org/10.6084/m9.figshare.12420824.v1). At 8 wk old, mice were randomized to stay on the same low-fat diet or to be fed high-fat diet (45% kcal fat, Research Diets D01060502; Supplemental Table S1). Food intake and body weight were measured weekly between Zeitgeber time (ZT) 9–12 (where ZT0 is lights on and ZT12 is lights off). Mice that lost >10% body weight in any 1-wk time span were removed from the experiment. Cumulative caloric intake was determined by summating the total number of calories consumed during the 7 wk of the experiment. Food consumption in grams was converted to kcal to permit comparison between low-fat diet (LFD: 3.85 kcal/g metabolizable energy) and high-fat diet (HFD: 4.73 kcal/g metabolizable energy) groups. At 14 wk old, mice were fasted for 8 h during the light phase (beginning at ZT2-4). Then they were euthanized by cervical dislocation and decapitation at ZT10-12, and blood glucose (Accu-Chek Aviva) and body composition (Echo MRI-100) were measured. All experiments were submitted to and approved by the University of Kentucky Institutional Animal Care and Use Committee (Protocol 2015-2211).

Eating Behavior Rhythm Recording and Analysis

Infrared video cameras (HD 48Led 940 nm Outdoor CMOS 800TVL IR-Cut Dome camera waterproof IF CCTV) connected to an MPX HD 1080p Security System DVR (Lorex) were used to continuously record eating behavior. Eating behavior was recorded during the first 2 wk and during the final week of the experiment. An observer watched the video and indicated whether the mouse was eating (yes is "1" and no is "0") in 1-min bins, as previously described (18). Oriana software (Oriana 4.0; Kovach Computing Services, Wales, UK) was used to make circular histograms and perform circular statistics at 1 wk of low-fat diet feeding (LFD), after 1 wk of high-fat diet feeding (short-term HFD), and after 6 wk of high-fat diet feeding (long-term HFD). Each circular histogram shows the distribution of eating events across one 24-h day. Circular statistics were used to determine if the eating behavior was uniformly distributed ($P < 0.05$ indicates that eating behavior was nonuniformly distributed and a daily rhythm was therefore present), and the vector of the distribution. We defined the length and direction of a vector as the amplitude and phase, respectively, of the eating behavior rhythm. If the eating behavior events were uniformly distributed across the day ($P \geq 0.05$), then we designated the amplitude as 0 and there was no value for phase. Most obesity-prone mice had arrhythmic eating behavior during HFD feeding and thus no values for phase, so we did not analyze eating behavior rhythm phase.

Locomotor Activity Rhythm Recording and Analysis

Passive infrared sensors (PIR motion sensor, product ID189, Adafruit) were used to continuously record general locomotor activity for the entire experiment. Single-plotted actograms (6-min bins, scaled setting) of locomotor activity were made using ClockLab software (Actimetrics). Oriana software was used to make circular histograms and perform circular statistics at 1 wk of LFD, after 1 wk of short-term HFD, and after 6 wk of long-term HFD. Circular statistics were used to determine whether locomotor activity was uniformly distributed ($P < 0.05$ indicates that activity was nonuniformly distributed and a daily rhythm was therefore present), and the vector of the distribution. We defined the length and direction of a vector as the amplitude and phase, respectively, of the activity rhythm. All mice had rhythmic activity ($P < 0.05$) during LFD and HFD feeding.

Statistical Analyses

First, we analyzed the effects of diet on each outcome separately for each strain. Second, we compared the effects of diet on each variable between obesity-prone strains (C57BL/6J and 129X1/SvJ) and obesity-resistant (SWR/J and BALB/cJ) strains. These approaches are described for each outcome below. Analyses were performed using SPSS software version 26.0. Significance was ascribed at $P < 0.05$ unless otherwise noted. Data are presented as means ± 95% confidence interval. For repeated-measures (RM) ANOVA demonstrating a violation of the sphericty assumption, Greenhouse–Geisser's correction was applied to the degrees of freedom.

Two-way repeated-measures ANOVA models (time × diet) were used to analyze the effects of diet on body weight over time for each strain separately. To analyze the obesity propensity of the male mice, we used a three-way repeated-measures ANOVA model (time × diet × group) to analyze the effects of diet on body weight over time for the obesity-prone strains (C57BL/6J and 129X1/SvJ) compared with the obesity-resistant strains (SWR/J and BALB/cJ). For post hoc analysis, we used two-group t tests to compare body weight between obesity-prone and obesity-resistant strains at each time point with Bonferroni-adjusted significance level = 0.006.

Unpaired two-tailed Student's t tests were used to determine whether high-fat diet consumption, compared with low-fat diet, affected adiposity, fasting blood glucose, cumulative caloric intake, and cumulative activity for each strain, unless the data were not normally distributed or had unequal variance, in which case the Mann–Whitney test was used. The effects of diet on adiposity, fasting blood glucose, cumulative caloric intake, and cumulative activity between

obesity-prone and obesity resistant strains were analyzed by two-way ANOVA.

One-way repeated-measures ANOVA was used to determine whether diet affected the amplitude of the eating behavior rhythm and the amplitude or phase of the locomotor activity rhythm for each strain (post hoc Dunnett's with LFD as control). To determine whether eating behavior rhythm amplitude and locomotor activity rhythm amplitude and phase differed between obesity-prone and obesity-resistant male mice, two-way repeated-measures ANOVA models (time × group) were used. For post hoc analysis, two-group *t* tests were used to compare eating behavior rhythm amplitude and activity rhythm amplitude between obesity-prone and obesity-resistant strains at each time point with Bonferroni-adjusted significance level = 0.017.

RESULTS

High-Fat Diet Feeding Differentially Affects Metabolism in Obesity-Prone and Obesity-Resistant Inbred Strains of Male Mice

We first examined the effects of high-fat feeding on body weight and blood glucose in strains of mice that were previously characterized as obesity prone (C57BL/6J and 129X1/SvJ) or obesity resistant (BALB/C and SWR/J). Male C57BL/6J mice gained weight at a higher rate when fed HFD compared with LFD (Fig. 1*A*; RM ANOVA time × diet, $F(2,49) = 25.45$, $P < 0.001$). HFD consumption also increased adiposity (Fig. 1*B*; $U = 0$, $P < 0.001$) and fasting blood glucose (Fig. 1*C*; $t_{28} = -2.52$, $P = 0.02$) compared with LFD feeding in C57BL/6J mice. Likewise, male 129X1/SvJ mice gained weight at a higher rate when fed HFD compared with LFD (Fig. 1*D*; RM ANOVA time × diet, $F(2,46) = 61.96$, $P < 0.001$) and had greater adiposity (Fig. 1*E*; $U = 0$, $P < 0.001$), but fasting blood glucose was not significantly different (Fig. 1*F*; $t_{20} = -1.89$, $P = 0.08$). Male SWR/J mice gained weight at a higher rate when fed HFD compared with LFD (Fig. 1*G*; RM ANOVA time × diet, $F(3,67) = 6.96$, $P < 0.001$), but adiposity (Fig. 1*H*; $U = 36$, $P = 0.07$) and fasting blood glucose (Fig. 1*I*; $U = 48$, $P = 0.28$) were not significantly altered by HFD feeding. Male BALB/cJ mice gained weight at a higher rate when fed HFD compared with LFD (Fig. 1*J*; RM ANOVA time × diet, $F(2,47) = 9.51$, $P < 0.001$) and had greater adiposity (Fig. 1*K*; $U = 18$, $P = 0.006$), but fasting blood glucose was not significantly altered (Fig. 1*L*; $t_{19} = 1.90$, $P = 0.07$).

We next determined whether HFD differentially affected metabolic parameters in obesity-prone and obesity-resistant strains of male mice (Fig. 1, *M*–*O*). Obesity-prone C57BL/6J and 129X1/SvJ male mice gained weight at a higher rate than obesity-resistant SWR/J and BALB/cJ male mice when fed HFD compared with LFD (Fig. 1*M* and Supplemental Table S2; RM ANOVA time × diet × group, $F(2,225) = 9.27$, $P < 0.001$). Obesity-prone mice weighed more than obesity-resistant mice during 6 wk of HFD feeding (post hoc: two-group *t* test at each time point, $P < 0.001$). Adiposity was also greater in obesity-prone compared with obesity-resistant male mice fed HFD compared with LFD (Fig. 1*N* and Supplemental Table S3; ANOVA diet × group, $F(1,95) = 16.47$, $P < 0.001$). In contrast, fasting blood glucose did not significantly differ between obesity-prone and obesity-resistant mice fed HFD compared with LFD (Fig. 1*O* and Supplemental Table S3 ANOVA diet × group, $F(1,94) = 1.89$, $P = 0.172$).

Since caloric intake and activity level contribute to body weight regulation, we next examined whether these factors were differentially affected by HFD feeding in the four strains of male mice (Fig. 2). We found that cumulative caloric intake was increased by HFD feeding compared with LFD, in C57BL/6J (Fig. 2*A*; $U = 5$, $P < 0.001$), 129X1/SvJ (Fig. 2*C*; $t_{20} = -9.57$, $P < 0.001$), SWR/J (Fig. 2*E*; $t_{22} = -6.05$, $P < 0.001$), and BALB/cJ (Fig. 2*G*; $t_{22} = -2.49$, $P = 0.02$) mice. Collectively, cumulative food intake differed between obesity-prone and obesity-resistant male mice fed HFD compared with LFD; however, the difference was driven by food intake during LFD and not HFD feeding (Fig. 2*I* and Supplemental Table S4; ANOVA diet × group, $F(1,96) = 10.31$, $P = 0.002$). We next measured total locomotor activity levels in each strain during LFD or HFD feeding. We found that total activity levels were not affected by HFD feeding in C57BL/6J (Fig. 2*B*; $t_{22} = 0.66$, $P = 0.51$), 129X1/SvJ (Fig. 2*D*; $t_{14} = 1.36$, $P = 0.20$), SWR/J (Fig. 2*F*, $U = 22$, $P = 0.80$), and BALB/cJ (Fig. 2*H*; $U = 71$, $P = 0.78$) male mice. Overall, total locomotor activity did not significantly differ between obesity-prone and obesity-resistant mice fed HFD compared with LFD (Fig. 2*J* and Supplemental Table S4; ANOVA diet × group, $F(1,75) = 0.03$, $P = 0.866$). These data suggest that caloric intake and activity level did not solely account for the distinct susceptibilities to diet-induced obesity in each strain.

Daily Rhythms of Eating Behavior Are Disrupted by High-Fat Feeding in Obesity-Prone, but Not in Obesity-Resistant, Male Mice

Previous studies have demonstrated that the timing of food intake is a determinant of HFD-induced obesity in C57BL/6 mice (19–21, 30, 31). Therefore, we next analyzed daily rhythms of eating behavior in the four strains of male mice during LFD and HFD consumption (Fig. 3 and Supplemental Figs. S1–S4). Similar to previous studies from our laboratory and from others, obesity-prone male C57BL/6J mice had high-amplitude daily rhythms of eating behavior that peaked during the night during LFD feeding (Fig. 3*A* and Supplemental Fig. S1) (16–18), but during short-term and long-term HFD feeding, the amplitude of the eating behavior rhythm was markedly reduced or arrhythmic (Fig. 3, *B*–*D*; RM ANOVA $F(2,6) = 7.32$, $P = 0.02$; post hoc $P < 0.05$ vs. LFD). Obesity-prone 129X1/SvJ male mice (Supplemental Fig. S2) also had high-amplitude eating behavior rhythms during LFD feeding (Fig. 3*E*) that gradually reduced in amplitude during short-term HFD (Fig. 3*F*) and then became low amplitude or arrhythmic during long-term HFD feeding (Fig. 3, *G* and *H*; RM ANOVA $F(2,4) = 21.56$, $P = 0.007$, post hoc $P < 0.02$ vs. LFD). In contrast, obesity-resistant SWR/J male mice (Supplemental Fig. S3) had high-amplitude eating behavior rhythms during LFD (Fig. 3*I*) and HFD feeding (Fig. 3, *J*–*L*; RM ANOVA $F(2,5) = 0.97$, $P = 0.44$). Obesity-resistant BALB/cJ male mice (Supplemental Fig. S4) also had high-amplitude eating behavior rhythms during LFD feeding (Fig. 3*M*) that did not differ significantly from HFD feeding (Fig. 3, *N*–*P*; RM ANOVA $F(2,5) = 0.41$, $P = 0.68$). The total number

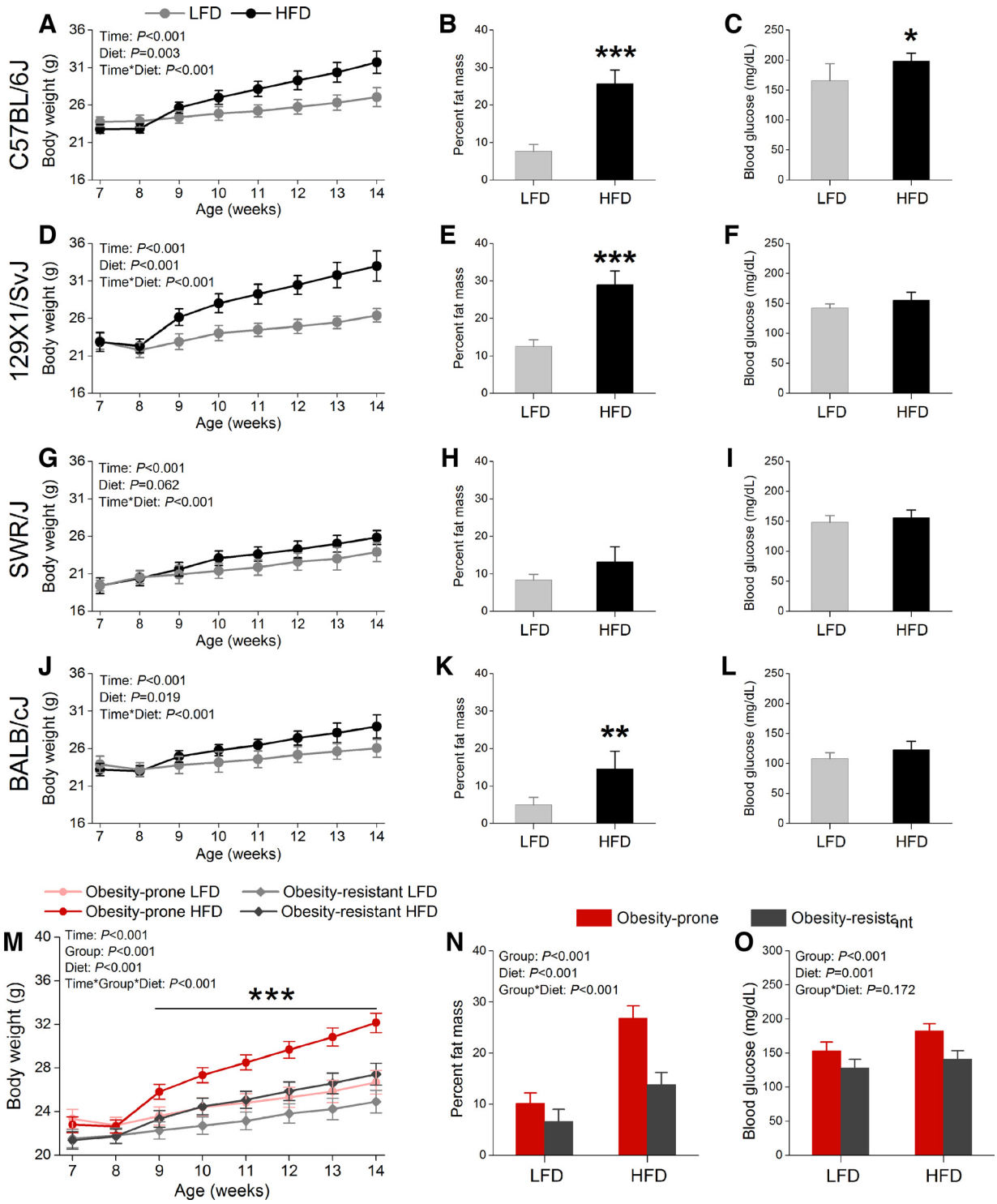

Figure 1. High-fat feeding causes obesity in obesity-prone, but not obesity-resistant, strains of male mice. Body weights were measured weekly from male C57BL/6J (*A*, n_{LFD} = 10; n_{HFD} = 20), 129X1/SvJ (*D*, n_{LFD} = 11; n_{HFD} = 11), SWR/J (*G*, n_{LFD} = 12; n_{HFD} = 12), and BALB/cJ (*J*, n_{LFD} = 11; n_{HFD} = 13) mice fed either low-fat diet (LFD) or high-fat diet (HFD) and analyzed with two-way repeated-measures ANOVA. All mice were fed LFD for 1 wk (7–8 wk old). HFD feeding began at 8 wk old in the HFD group. Percent fat mass (*B*, *E*, *H*, and *K*) and fasting blood glucose (*C*, *F*, *I*, and *L*) were measured in 14-wk-old C57BL/6J (*B* and *C*), 129X1/SvJ (*E* and *F*), SWR/J (*H* and *I*), and BALB/cJ (*K* and *L*) mice fed either LFD or HFD and were analyzed with Student's *t* tests, except *B*, *E*, *H*, *I*, and *K*, which were analyzed with Mann–Whitney tests. Body weights (*M*, prone n_{LFD} = 21 LFD, n_{HFD} = 31; resistant n_{LFD} = 23, n_{HFD} = 25) were compared between male obesity-prone (C57BL/6J and 129X1/SvJ) and obesity-resistant (SWR/J and BALB/cJ) mice using three-way repeated-measures ANOVA with post hoc two-group *t* tests at each time point (Bonferroni-adjusted significance level = 0.006). Adiposity (*N*, prone n_{LFD} = 21; n_{HFD} = 31 HFD; resistant n_{LFD} = 22, n_{HFD} = 23) and fasting blood glucose (*O*, prone n_{LFD} = 21, n_{HFD} = 31; resistant n_{LFD} = 22, n_{HFD} = 22) were compared between male obesity-prone and obesity-resistant mice using two-way ANOVA. Data are means ± 95% confidence interval. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

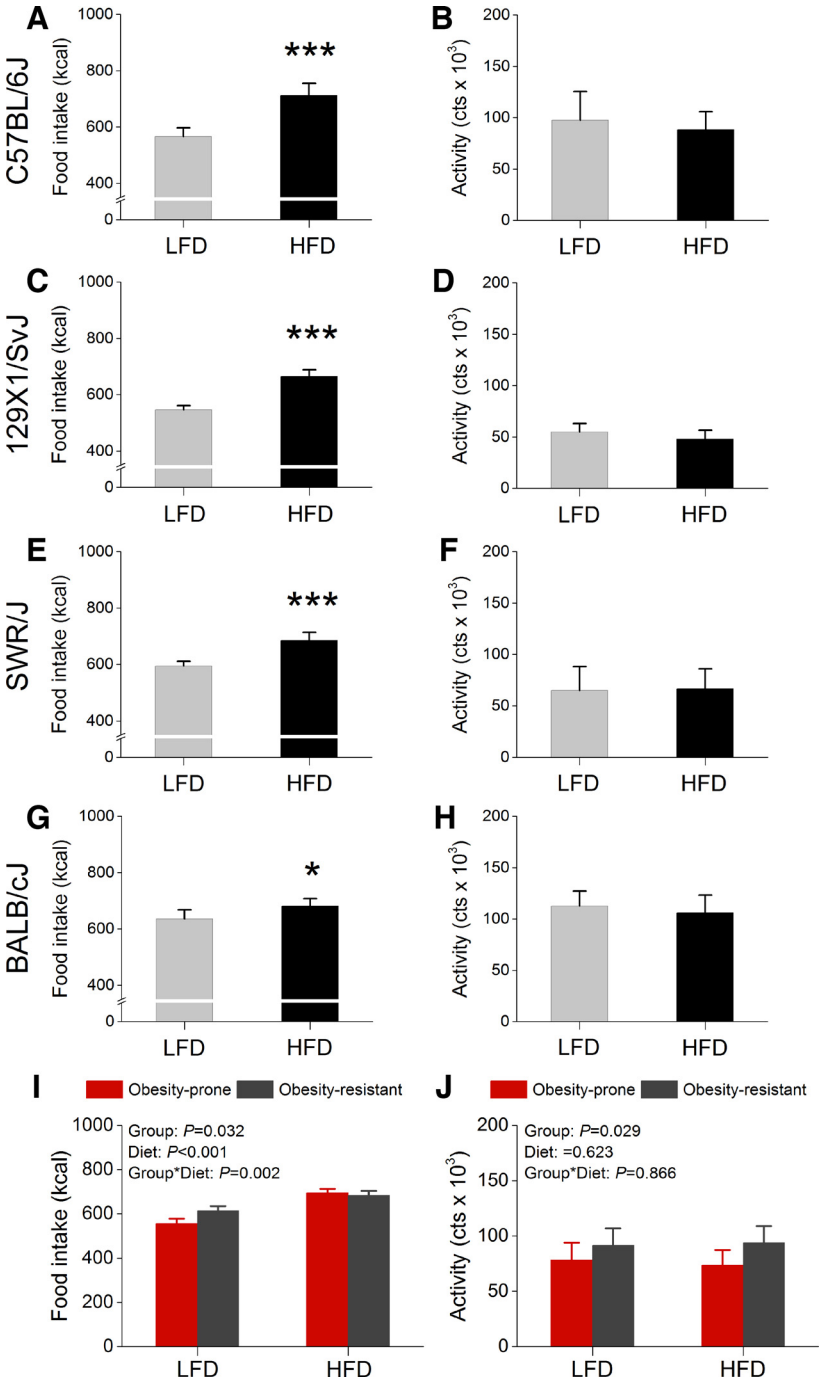

Figure 2. Food intake and activity do not differ between obesity-prone and obesity-resistant mice during high-fat feeding. Cumulative food intake was measured in male C57BL/6J (*A*, n_{LFD} = 10; n_{HFD} = 18), 129X1/SvJ (*C*, n_{LFD} = 11; n_{HFD} = 11), SWR/J (*E*, n_{LFD} = 12; n_{HFD} = 12), and BALB/cJ (*G*, n_{LFD} = 11; n_{HFD} = 13) mice fed either low-fat diet (LFD) or high-fat diet (HFD). Total locomotor activity counts were measured in male C57BL/6J (*B*, n_{LFD} = 10; n_{HFD} = 14), 129X1/SvJ (*D*, n_{LFD} = 8; n_{HFD} = 8), SWR/J (*F*, n_{LFD} = 7; n_{HFD} = 7), and BALB/cJ (*H*, n_{LFD} = 11; n_{HFD} = 12) mice. Locomotor activity counts could not be measured for some mice due to faulty infrared sensors. *A–J* were analyzed with Student's *t* tests, except *A*, *F*, and *H*, which were analyzed with Mann–Whitney tests. Cumulative food intake (*I*, prone n_{LFD} = 21, n_{HFD} = 29; resistant n_{LFD} = 23, n_{HFD} = 25) and activity (*J*, prone n_{LFD} = 18; n_{HFD} = 22; resistant n_{LFD} = 18, n_{HFD} = 19) were compared between male obesity-prone (C57BL/6J and 129X1/SvJ) and obesity-resistant (SWR/J and BALB/cJ) mice using two-way ANOVA. Data are means ± 95% confidence interval. *$P < 0.05$, ***$P < 0.001$.

of eating events was reduced during consumption of calorie dense HFD in all four strains (Supplemental Fig. S9).

We next determined whether HFD feeding differentially affected eating behavior rhythms in obesity-prone and obesity-resistant strains of male mice (Fig. 3*Q*). The change in eating behavior rhythm amplitude between LFD and HFD feeding differed in obesity-prone and obesity-resistant strains of male mice (Supplemental Table S5; RM ANOVA time × group, $F(2,52) = 8.10$, $P = 0.001$). The amplitude of the eating behavior rhythm was significantly reduced during long-term HFD feeding (post hoc: two-group *t* test at each time point, $P = 0.001$) but not during LFD ($P = 0.235$) or short-term HFD ($P = 0.034$) feeding, in obesity-prone compared with obesity-resistant males.

High-Fat Feeding Does Not Differentially Affect Activity Rhythms in Obesity-Prone and Obesity-Resistant Male Mice

We next analyzed the effects of short-term and long-term HFD feeding on locomotor activity rhythms in each strain of male mice. In obesity-prone C57BL/6J male mice (Fig. 4, *A–D*, and Supplemental Fig. S5), HFD feeding did not significantly

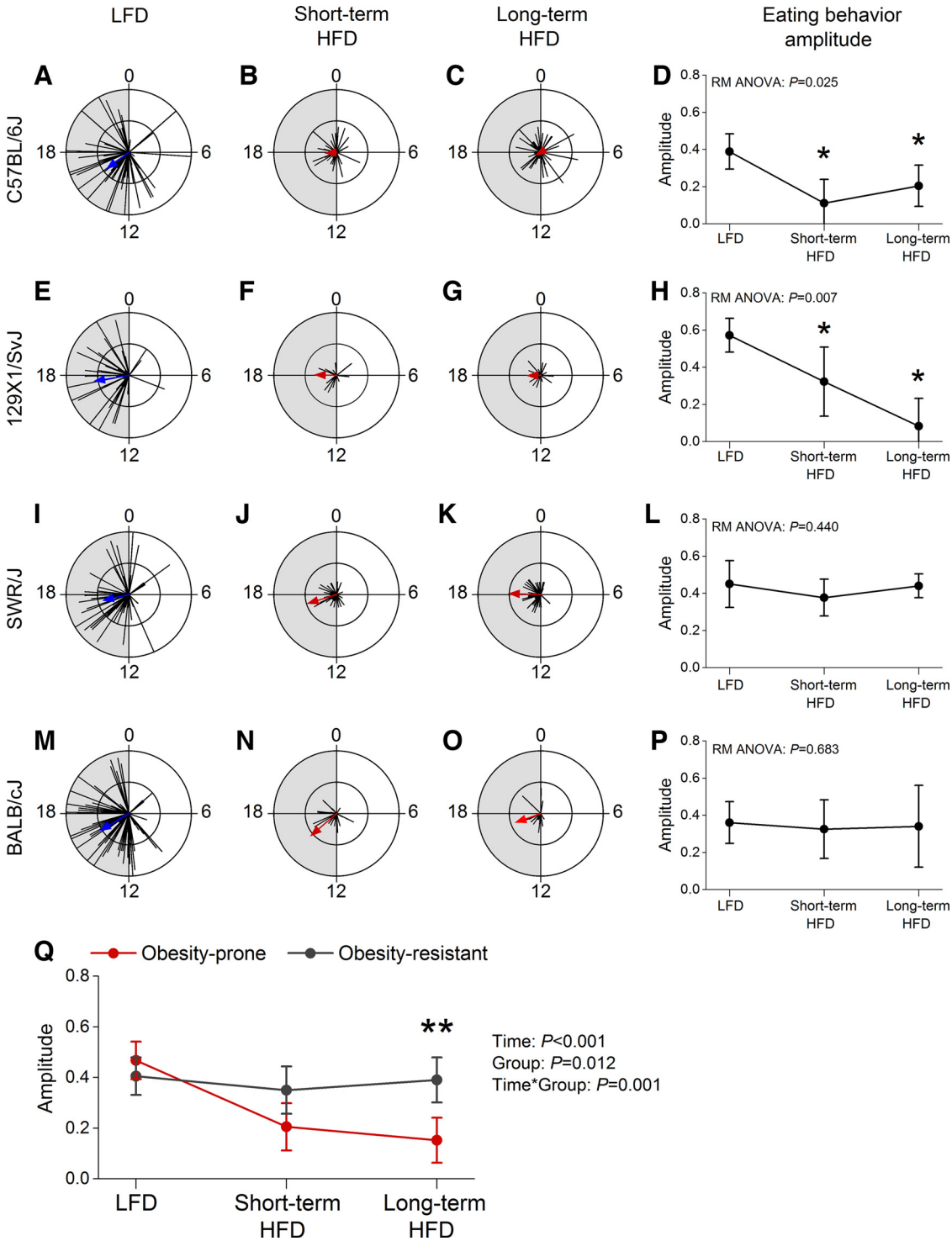

Figure 3. High-fat feeding disrupts the eating behavior rhythm in obesity-prone, but not obesity-resistant, male mice. Representative circular histograms of eating behavior (10-min bins) during LFD (*A*, *E*, *I*, and *M*), short-term HFD (*B*, *F*, *J*, and *N*), and long-term HFD (*C*, *G*, *K*, and *O*) for C57BL/6J (*A*–*C*), 129X1/SvJ (*E*–*G*), SWR/J (*I*–*K*), and BALB/cJ (*M*–*O*) male mice (scale: *inner* circle, 0; *middle* circle, 5; *outer* circle, 10). Amplitudes (vector lengths) of the eating behavior rhythms of C57BL/6J (*D*, $n = 8$), 129X1/SvJ (*H*, $n = 6$), SWR/J (*L*, $n = 7$), and BALB/cJ (*P*, $n = 7$) male mice were analyzed with one-way repeated-measures ANOVA followed by Dunnett's post hoc vs. LFD group. *Q*: eating behavior rhythm amplitudes were compared between obesity-prone (C57BL/6J and 129X1/SvJ; $n = 14$) and obesity-resistant mice (SWR/J and BALB/cJ; $n = 14$) using two-way repeated-measures ANOVA (time × group) and post hoc two-group *t* tests. Data are means ± 95% confidence interval. $^{*}P < 0.05$, $^{**}P = 0.001$.

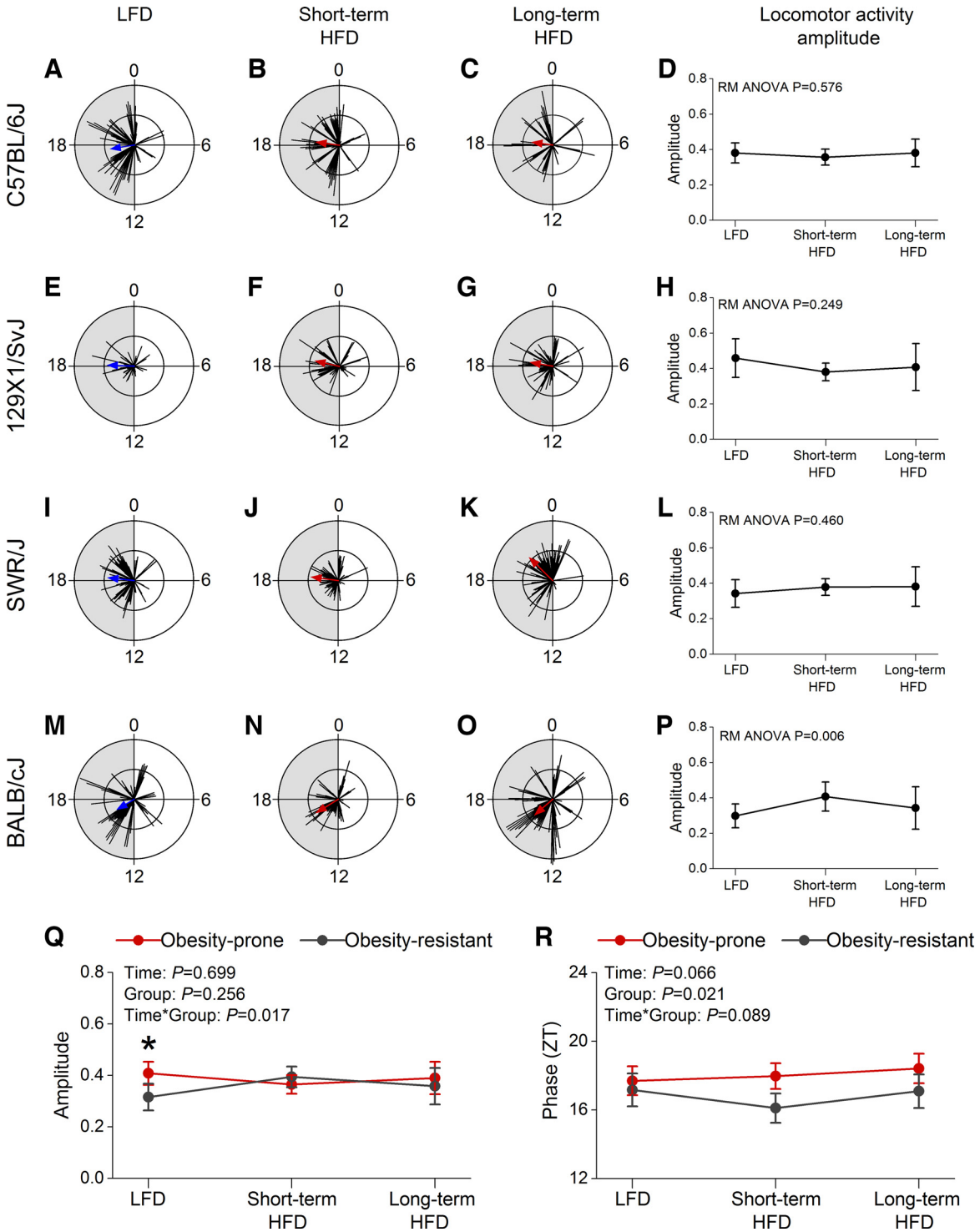

Figure 4. Locomotor activity rhythms do not differ between obesity-prone and obesity-resistant mice during high-fat feeding. Representative circular histograms of locomotor activity (10-min bins) during LFD (*A*, *E*, *I*, and *M*), short-term HFD (*B*, *F*, *J*, and *N*), and long-term HFD (*C*, *G*, *K*, and *O*) for C57BL/6J (*A–C*), 129X1/SvJ (*E–G*), SWR/J (*I–K*), and BALB/cJ (*M–O*) male mice (scale: *inner* circle, 0; *middle* circle, 30; *outer* circle, 60). Amplitudes (vector lengths) of the activity rhythms of C57BL/6J (*D*, $n = 14$), 129X1/SvJ (*H*, $n = 8$), SWR/J (*L*, $n = 7$), and BALB/cJ (*P*, $n = 10$) male mice were analyzed with one-way repeated-measures ANOVA followed by Dunnett's post hoc vs. LFD group. Locomotor activity rhythm amplitudes (*Q*) and phases (*R*) were compared between obesity-prone (C57BL/6J and 129X1/SvJ; $n = 22$) and obesity-resistant mice (SWR/J and BALB/cJ; $n = 17$) using two-way repeated-measures ANOVA model (time × group). Data are means ± 95% confidence interval. $^*P < 0.05$.

affect the amplitude (Fig. 4*D*, RM ANOVA $F(2,12) = 0.579$, $P = 0.576$) of the activity rhythm. The phase of the activity rhythm (Supplemental Fig. S10*A*) in C57BL/6J males was not affected by short-term HFD feeding (RM ANOVA $F(2,12) = 9.62$, $P = 0.003$, post hoc $P = 0.993$ vs. LFD) but was different from LFD after long-term HFD feeding (post hoc $P = 0.002$ vs. LFD). In obesity-prone 129X1/SvJ males (Fig. 4, *E–H*, and Supplemental Fig. S6), the amplitude (Fig. 4*H*; RM ANOVA $F(2,6) = 1.77$, $P = 0.249$) and the phase (Supplemental Fig. S10*B*; RM ANOVA $F(2,6) = 5.41$, $P = 0.050$) of the activity rhythm were not significantly affected by HFD feeding. Likewise, in obesity-resistant SWR/J males (Fig. 4, *I–L*, and Supplemental Fig. S7), the amplitude (Fig. 4*L*; RM ANOVA $F(2,5) = 0.91$, $P = 0.460$) and phase (Supplemental Fig. S10*C*; RM ANOVA $F(2,5) = 4.87$, $P = 0.067$) of the activity rhythm were not significantly altered by HFD feeding. In obesity-resistant BALB/cJ male mice (Fig. 4, *M–P*, and Supplemental Fig. S8), HFD feeding affected the amplitude of the activity rhythm (Fig. 4*P*; RM ANOVA $F(2,8) = 10.09$, $P = 0.006$) and there was a trend for the amplitude to increase during short-term HFD feeding compare to LFD (post hoc $P = 0.054$ vs. LFD). The phase of the activity rhythm was not significantly affected by HFD feeding in BALB/cJ males (Supplemental Fig. S10*D*; RM ANOVA $F(2,8) = 2.71$, $P = 0.126$).

We next determined whether HFD feeding differentially affected activity rhythms in obesity-prone and obesity-resistant strains of male mice (Fig. 4, *Q* and *R*). The change in activity rhythm amplitude between LFD and HFD feeding differed in obesity-prone and obesity-resistant strains of male mice (Fig. 4*Q* and Supplemental Table S6; RM ANOVA time × group, $F(2,74) = 4.34$, $P = 0.017$). However, the activity rhythm amplitude differed between obesity-prone and obesity-resistant mice only during LFD feeding (post hoc: two-group *t* test at each time point, $P = 0.009$) and not during short-term HFD ($P = 0.264$) or long-term HFD ($P = 0.496$) feeding. The change in the phase of the activity rhythm during LFD and HFD feeding did not significantly differ between obesity-prone and obesity-resistant male mice (Fig. 4*R* and Supplemental Table S6; RM ANOVA time × group, $F(2,74) = 2.68$, $P = 0.089$).

DISCUSSION

Obesity is epidemic in Western countries where calorie-dense, high-fat diets are regularly consumed (32, 33). The circadian system has emerged as a critical player in regulating HFD-induced obesity (14). HFD feeding has widespread effects on the circadian system and at all levels of organization, from behavior to physiology to gene expression, in male mice (17, 18, 34, 35). The consumption of HFD disrupts the daily eating rhythm, and using time-restricted feeding to restore the high-amplitude feeding rhythm inhibits diet-induced obesity in male C57BL/6J mice (17, 18, 21). Clinical studies have also shown that targeting the daily eating rhythm may be a successful therapeutic for obesity or other metabolic dysfunctions (36–38). Because of the striking effect of HFD feeding on the eating rhythm and because regulation of the eating rhythm is linked to obesity in C57BL/6J male mice, we hypothesized that susceptibility or resistance of the daily eating rhythm to disruption during HFD feeding might correlate with propensity or resistance, respectively, to developing diet-induced obesity. To test this hypothesis, we systematically compared the effects of HFD feeding on daily rhythms in obesity-prone and obesity-resistant strains of male mice.

Consistent with prior studies, we found that male obesity-prone C57BL/6J and 129X1/SvJ mice gained weight at a higher rate than male obesity-resistant SWR/J and BALB/cJ mice (23, 25, 26, 28). In addition, both obesity-prone and obesity-resistant male mice consumed more calories during HFD feeding compared with LFD feeding despite differences in body weight gain. Prior studies also found that strain differences in propensity to develop obesity during HFD feeding could not be explained by differences in HFD energy intake (22, 27–29). In addition, in both obesity-prone and obesity-resistant male mice, overall levels of locomotor activity were not affected by HFD feeding. Thus, strain-specific responses to HFD were not simply attributed to differences in energy intake and activity.

This study is the first to systematically compare the effects of HFD feeding on the daily rhythm of eating behavior in obesity-prone and obesity-resistant male mice. We found that HFD disrupted the eating behavior rhythm in obesity-prone but not in obesity-resistant strains of male mice. Several previous studies from our laboratory and others, as well as this study, showed that HFD feeding reduced the amplitude of the daily eating rhythm in male C57BL/6J mice (16–18, 39). In this study, we showed that the amplitude of the eating behavior rhythm was also markedly decreased by HFD feeding in obesity-prone 129X1/SvJ male mice. In contrast, the amplitudes of the eating behavior rhythms in obesity-resistant SWR/J and BALB/cJ males were not altered by HFD feeding. Our results are consistent with a recent study that showed that C57BL/6J, but not BALBc/J, male mice consumed more calories during the daytime inactive phase during HFD feeding compared with LFD feeding (22). In sum, we find that the robustness of the eating rhythm during HFD feeding negatively correlates with propensity to HFD-induced obesity. The strains of male mice that are resistant to diet-induced obesity also maintain robust, high-amplitude eating behavior rhythms during HFD feeding. These data suggest that maintenance of high-amplitude eating behavior rhythms during HFD feeding may be a mechanism that protects male mice from diet-induced obesity (Fig. 5).

We also examined the effects of HFD feeding on locomotor activity rhythms in obesity-prone and obesity-resistant male mice. Overall, there were no significant differences between obesity-prone and obesity-resistant strains of male mice in the amplitude or phase of the locomotor activity rhythm. Studies from our laboratory and others' have investigated the effects of HFD feeding on locomotor activity rhythms in male obesity-prone C57BL/6J mice. These studies found either no effect or small effects of HFD on the amplitude and phase of activity rhythms in male C57BL/6J mice entrained to light-dark cycles (17, 18, 35, 39). Some of these prior studies measured wheel-running activity, while others measured general locomotor activity, and the diets varied between studies, which could account for the discrepant findings. The novel approach in this study was to study both obesity-prone and obesity-resistant male mice under identical conditions. We measured daily rhythms of general locomotor

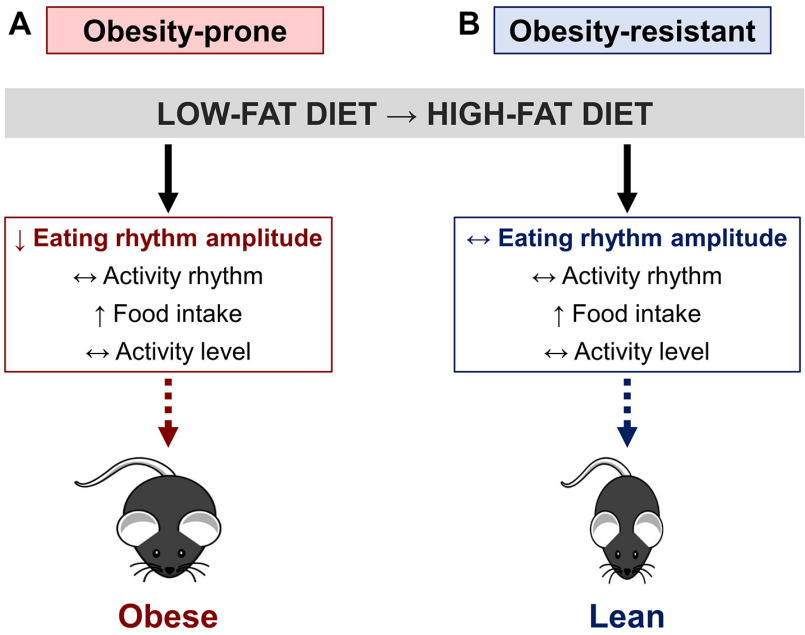

Figure 5. Proposed model of circadian regulation of diet-induced obesity in different inbred strains of male mice. In obesity-prone strains of male mice (*A*), the amplitude of the daily rhythm of eating behavior is markedly decreased by high-fat diet (HFD) feeding. In contrast, in obesity-resistant strains of male mice (*B*), HFD feeding does not alter the amplitude of the daily eating behavior rhythm. The activity rhythm is not differentially affected by HFD feeding in obesity-prone or obesity-resistant male mice. In both obesity-prone and obesity-resistant male mice, HFD feeding increases food intake but does not alter activity levels. We propose that regulation of the eating behavior amplitude is a mechanism that regulates propensity or resistance to diet-induced obesity. ↑, ↓, and ↔ indicate increase, decrease, or no change, respectively, during HFD feeding compared with low-fat diet (LFD) feeding.

activity to avoid the confounding effects of exercise from wheel-running activity. In this study, we found that HFD does not differentially affect daily activity rhythms in obesity-prone and obesity-resistant strains of male mice.

A limitation of this study is that we studied only male mice. We have previously shown that HFD feeding differentially affects daily rhythms in male and female mice (18, 40). In contrast to male mice, the eating behavior rhythm is robust in C57BL/6J female mice during HFD feeding. Circulating estradiol is required to protect daily rhythms from disruption by HFD feeding in C57BL/6J female mice (31). Thus, future studies should investigate the interplay among strain, circadian rhythms, and estrogens in regulating diet-induced obesity in female mice.

Genetic background has also been shown to regulate propensity for circadian gene mutant mice to develop diet-induced obesity. *Clock*$^{\Delta 19}$ mutant mice have a dominant negative mutation in the *Clock* gene and thus disrupted molecular timekeeping and circadian behavior (41, 42). Diet-induced obesity was exacerbated in male *Clock*$^{\Delta 19}$ mice on the C57BL/6J background compared with wild-type mice (43). In contrast, the *Clock*$^{\Delta 19}$ mutation attenuated diet-induced obesity in males on a JcI:ICR genetic background (44). These studies highlight the importance of considering the impact of genetic background in studies of circadian regulation of metabolism.

Genetic background could impact the circadian regulation of eating behavior via several different mechanisms. HFD feeding could differentially affect nutrient sensing in obesity-prone and obesity resistant mice. We speculate that nutrient sensing may be differentially altered by HFD, since the eating behavior rhythm is rapidly disrupted by HFD feeding and rapidly reversed upon return to LFD in obesity-prone male mice (16–18). Many nutrient sensors, such as AMPK, NAD^+, PPARs, and leptin interact with circadian clocks, are impacted by HFD feeding, and can alter eating behavior (45, 46). Obesity-prone and obesity-resistant mouse strains could also have differential HFD responses in neural substrates in the gut and brain. The hindbrain nucleus of the solitary tract (NTS) is a candidate neural locus, since it receives afferents from the gut and modulates feeding behavior, it contains an endogenous circadian clock, and its responsiveness to peripheral signals is altered by HFD feeding (45, 47–49). Future studies could investigate the impact of HFD feeding on circadian rhythms of nutrient sensors in peripheral and central tissues in obesity-prone and obesity-resistant mice.

Perspectives and Significance

Previous studies have investigated the genetic basis of HFD-induced obesity and insulin resistance using inbred mouse strains (50–53). Recent studies have revealed the importance of circadian rhythms in regulating obesity, which led us to investigate whether rhythms are differentially affected in inbred mouse strains. We found a striking difference between male obesity-prone and obesity-resistant mice in the eating behavior rhythm during HFD feeding. Future studies can use molecular genetics to identify novel genes and alleles that regulate responses of circadian eating behavior to high-fat diet.

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DISCLOSURES

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

DISCLAIMERS

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

AUTHOR CONTRIBUTIONS

J.S.P. conceived and designed research; T.N.B., J.S.P., O.O., L.A.A., C.R.R., E.P.K., J.D.L., and J.M.C. performed experiments; T.N.B., J.S.P., O.O., L.A.A., C.R.R., E.P.K., J.D.L., J.M.C., F.L. and E.S. analyzed data; T.N.B. and J.S.P. interpreted results of experiments; T.N.B. and J.S.P. prepared figures; T.N.B. and J.S.P. drafted manuscript; T.N.B. and J.S.P. edited and revised manuscript; T.N.B., J.S.P., O.O., L.A.A., C.R.R., E.P.K., J.D.L., J.M.C., F.L. and E.S. approved final version of manuscript.

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