Why Do Mice Overeat High-Fat Diets? How High-Fat Diet Alters the Regulation of Daily Caloric Intake in Mice

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Objective: *Ad libitum* high-fat diets (HFDs) spontaneously increase caloric intake in rodents, which correlates positively with weight gain. However, it remains unclear why rodents overeat HFDs. This paper investigated how changing the proportion of diet that came from HFDs might alter daily caloric intake in mice.

Methods: Mice were given 25%, 50%, or 90% of their daily caloric need from an HFD, along with *ad libitum* access to a low-fat rodent chow diet. Food intake was measured daily to determine how these HFD supplements impacted total daily caloric intake. Follow-up experiments addressed the timing of HFD feeding.

Results: HFD supplements did not alter total caloric intake or body weight. In a follow-up experiment, mice consumed approximately 50% of their daily caloric need from an HFD in 30 minutes during the light cycle, a time when mice do not normally consume food.

Conclusions: An HFD did not disrupt regulation of total daily caloric intake, even when up to 90% of total calories came from the HFD. However, HFDs increased daily caloric intake when provided *ad libitum* and were readily consumed by mice outside of their normal feeding cycle. *Ad libitum* HFDs appear to induce overconsumption beyond the mechanisms that regulate daily caloric intake.

Introduction

Obesity is a leading public health challenge in the United States (1). Even after being recognized as a national epidemic in 1999, obesity rates rose steadily throughout the beginning of the 21st century, and currently more than 30% of Americans have obesity (2,3). Although the causes of the obesity epidemic are complex, increases in food intake appear to be at least partly responsible (4). Across the globe, increases in food production correlate with increases in obesity rates of different countries, supporting the link between food intake and obesity rate (5,6). In addition, high-fat diet (HFD) access causes a spontaneous increase in daily caloric intake in rodents, which correlates positively with weight gain across individuals (7,8). Although *ad libitum* access to an HFD increases daily caloric intake and leads to weight gain in rodents (9), the reasons for this are not fully understood. Here, we sought to understand how an HFD alters the regulation of daily caloric intake in mice.

There are generally thought to be two systems that govern food intake: homeostatic hunger systems and hedonic reward systems (10). Homeostatic hunger systems involve endocrine and neural feedback signals of hunger and satiety that work to maintain a body weight “set point” (11). Dysregulation of this homeostatic control may contribute to obesity (12). For example, melanocortin 4 receptors (MC4Rs) are expressed in regions of the brain that control autonomic and endocrine functions (13), and inhibition of MC4R function increases food intake (14). In addition, leptin is secreted by adipocytes to promote satiety. Mutations that disrupt the production of or receptors for leptin result in hyperphagia and obesity in humans (15) and rodents (16). However, mutations in leptin, MC4R, and other genes involved in homeostatic feedback control are rare in humans (13,17) and do not account for HFD-induced obesity in wild-type rodents. Animals maintained on an HFD also do not have deficits in leptin signaling but instead have high circulating leptin
levels (18). Therefore, deficits in homeostatic hunger signaling do not appear sufficient to explain how an HFD alters the regulation of daily caloric intake in rodents.

Instead, we hypothesized that hedonic systems are responsible for HFD-induced overconsumption and consequent weight gain. To test this hypothesis, we provided mice with varying proportions of an HFD, equal to 25%, 50%, or 90% of their daily caloric intake. They were also given ad libitum access to their regular low-fat chow diet. We predicted that (1) if homeostatic systems were not adequately engaged by HFDs, mice would overconsume the low-fat chow and their total daily caloric intake would increase, but (2) if these systems were engaged by HFDs, total daily caloric intake would remain stable, regardless of the percentage of their diet that came from HFDs. This second possibility was in line with our hypothesis that hedonic systems are responsible for overconsumption of HFDs and was supported by the data; even when mice received up to 90% of their daily calories from an HFD, neither total daily caloric intake nor weight increased. We confirmed that these same mice would overconsume an HFD, and gain weight when an HFD was provided ad libitum. We concluded that ad libitum HFDs lead to overconsumption because of mechanisms outside of the homeostatic regulation of total daily caloric intake. These likely include those that govern hedonic food reward. In a final experiment, we confirmed that mice would eat nearly 50% of their daily caloric need from an HFD in a single 30-minute period during the light cycle, a time when mice normally do not eat. Assessing the relative contributions of homeostatic and hedonic regulation of food intake may inform strategies to treat human obesity.

**Methods**

**Animals**

All mice (C57/BL6 background) were individually housed under standard conditions (12-hour light/dark cycle, 23-25°C). Mice were weight matched and randomized to groups before all experiments involving multiple measurements. All procedures were in accordance with guidelines from the Animal Care and Use Committee of the National Institute on Diabetes and Digestive and Kidney Diseases.

**Diets**

Diets included low-fat chow diet (3.10 kcal/g with 29% energy derived from protein, 13% from fat, and 56% from carbohydrate; 5001 Laboratory Rodent Diet; LabDiet, St. Louis, Missouri), 60% HFD (5.24 kcal/g with 20% energy derived from protein, 60% from fat, and 20% from carbohydrate; D12492; Research Diets, Inc., New Brunswick, New Jersey), or homemade diets of varying fat percentages of roughly 20%, 30%, 40%, 50%, 60%, or 70% (percentages were determined by kilocalorie). Homemade diets were made by mixing low-fat chow diet with lard (46% of the fat being saturated fat and 50% unsaturated fat) or Crisco vegetable shortening (The J.M. Smucker Co., Orrville, Ohio) (29% of the fat being saturated fat and 65% unsaturated fat) using a blender.

**Food intake measurements**

Food intake measurements were obtained by manually weighing food. Mice were given food in Rodent Cafes (OYC Americas, Inc., Vista, California), which were weighed every 24 hours to derive food consumption. All measurements were converted to calories to compare between different diets.

**Schedule of diets**

For ad libitum high-fat feeding experiments, mice received an ad libitum HFD (D12492) for 9 weeks. Intake was measured with Rodent Cafes every 2 to 3 days.

For homemade diet experiments, diets were delivered in a randomized crossover design in which each mouse was tested with two homemade diets of the same fat source (lard or vegetable shortening). The repeated test design was employed to limit the number of animals used, and the two measurements were considered independent for the analysis of variance (ANOVA).

For high-fat supplement experiments, mice were provided premeasured HFD supplements of 25%, 50%, or 90% of their estimated normal total daily caloric intake (estimated at 10 kcal for all mice). In a separate Rodent Café, mice were concurrently provided access to ad libitum chow that was weighed daily, 2 hours into the light cycle. Each HFD supplement was provided for 1 week, and these tests were conducted back to back, starting with 1 week of the 25% HFD to habituate mice to the diet, followed by 25%, 90%, then 50% HFD supplement phases. HFD supplements were provided daily, 2 hours into the light cycle. Mice were weighed once each week. Following these supplement experiments, mice were given an ad libitum HFD for 5 weeks. Food intake was measured daily for the first week of this phase.

For intermittent feeding, mice were given an HFD supplement such that 50% of their daily caloric needs were given via HFD for 24 days, along with ad libitum access to chow. For the first 16 days, mice were provided access to HFD using the same protocol as the premeasured supplements. The first 8 days were considered habituation while the data from days 9 through 16 were analyzed. Mice were then provided the same amount of HFD but dispensed intermittently (described in “Intermittent HFD dispensing”). The amount of 800 mg of HFD, approximately 8% of their caloric need, was premeasured and dispensed every 4 hours, totaling 50% of their caloric intake (six dispensations per day). These supplement pieces were large enough to visualize in the bedding, and we never observed any HFD left over each day. Chow consumption was also measured daily 2 hours into the light cycle, after which HFD dispensing started.

For experiments measuring food intake in the light cycle, mice were given 1 g (3.10 kcal) of chow or 1 g (5.24 kcal) of HFD for 30 minutes, 3 hours after the light cycle. Mice were randomized to receive either chow or HFD. On the following day, mice were given another 30 minutes of access to the other diet such that each mouse was tested on both diets.

**Open field activity**

The average speed (centimeters per second) of mice was assessed by using PhenoTyper cages (30 × 30 cm; Noldus Information Technology, Inc., Leesburg, Virginia) and EthoVision video analysis software (version 11; Noldus Information Technology) following 9 weeks of HFD. Briefly, mice were videotaped from above for 20 minutes, and the position of the mice was extracted from these videos. Velocity was calculated from position. Minutes 5 to 20 were used for analysis.

**Body composition and energy expenditure calculations**

Energy expenditure was determined using the following energy balance calculation (19,20):
Energy expenditure = Metabolized energy intake − (∆ fat mass + ∆ fat-free mass)

Body composition (fat and fat-free mass) was measured using 1H-NMR spectroscopy (EchoMRI-100H; Echo Medical Systems LLC, Houston, Texas), while metabolized energy intake was calculated from food intake measurements.

**Intermittent HFD dispensing**

We made a home cage-compatible device to drop premeasured HFD every 4 hours. Twelve wells to hold HFD were three-dimensionally printed and mounted onto a 24-hour clock movement (three-dimensional file available at https://hackaday.io/project/106587-snack-clock). The clock movement rotated the dividers and pushed food into the cage at 4-hour intervals.

**Statistical analysis**

Statistical analysis was performed using GraphPad Prism (version 6.07; GraphPad Software, Inc., La Jolla, California). Two-tailed Student t test, one-way repeated-measures ANOVA, or two-way repeated-measures ANOVA were used when appropriate and as stated. Sidak’s multiple comparison test was used for post hoc comparisons. Results were considered significant at an alpha of $P < 0.05$.

**Results**

We provided mice ($n = 16$) ad libitum access to an HFD for 9 weeks and measured food intake, weight, and body composition, from which we calculated energy expenditure (Figure 1) (19,20). Weight and total HFD intake over 9 weeks were positively correlated (Figure 1A) ($R^2 = 0.73$, $P < 0.0001$), indicating that weight gain was associated with caloric intake (8,21). Weight was also positively correlated with energy expenditure (Figure 1B) ($R^2 = 0.33$, $P < 0.05$), while movement in an open field arena did not correlate with weight (Figure 1C) ($R^2 = 0.04$, $P = 0.44$). Food intake and energy expenditure were also positively correlated (Figure 1D) ($R^2 = 0.77$, $P < 0.0001$). These results are consistent with the conclusion that an ad libitum HFD increases both total calorie intake and energy expenditure (21-23).

We next asked whether the fat content of the diet was sufficient to drive overconsumption. Mice were fed homemade diets containing a...
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range of fat (14%, 20%, 30%, 40%, 50%, 60%, or 70%) from either lard or vegetable shortening (Figure 2). Each diet exposure lasted 3 days and included a control group that was tested only on chow (n = 8). Experimental animals (N = 48) were tested in a randomized design such that each mouse was tested on two homemade diets. By two-way ANOVA, we detected a significant effect of fat percentage (F[6, 98] = 17.21; P < 0.0001) and fat source (F[1, 98] = 7.391; P = 0.0078) but no significant interaction between them (F[6, 98] = 0.764; P = 0.5999). Post hoc tests (Sidak’s multiple comparison test) revealed that all diets with added fat were associated with higher total caloric intake than chow (all P < 0.05; Figure 2). Despite the significant effect of fat source by ANOVA, post hoc tests did not reveal any significant difference between fat source in any individual diet composition (all P > 0.08).

There are multiple reasons why mice might overeat an HFD. It is possible that the diet does not adequately engage the homeostatic mechanisms regulating total daily caloric intake. Alternatively, it is possible that mice overconsume the diet in spite of these mechanisms. We examined this in a new group of mice (n = 24) that were given 25%, 50%, and 90% of their daily caloric need (estimated at 10 kcal) from HFD supplements for 1 week each (Figure 3A). These mice also had ad libitum access to their regular chow diet for the duration of the study. Relative to control mice (n = 8) that were maintained on chow across the entire experiment, mice receiving HFD supplements did not increase their total daily caloric intake. This supports the contention that calories from HFDs engage the regulatory mechanisms that govern daily total caloric intake (Figure 3B-3C). A repeated-measures one-way ANOVA revealed a significant interaction between the experimental group and preload amount (F[3, 90] = 7.569; P = 0.0001). Interestingly, this interaction was driven by the 25% and 50% (but not 90%) preload groups eating slightly less than the control group (Figure 3B-3C; Sidak’s multiple comparison test, P < 0.05). In addition, mice receiving HFD supplements did not gain weight during this experiment, supporting the conclusion that their caloric intake remained stable (Figure 3D; two-tailed t test, P = 0.73). Next, we provided these same mice with ad libitum access to an HFD and measured their total daily caloric intake along with their weights for 5 weeks. As expected, animals overate and gained weight when the HFD was provided ad libitum (Figure 4A-4C; P < 0.0001 for both comparisons).

One unique aspect of this experimental design was that the supplements were administered only once per day, whereas mice normally consume ad libitum diets throughout the day and night. To test whether the timing of dispensing affected our results, we designed a device that would droppremeasured HFD pieces into the cage at 4-hour intervals (Figure 5A-5C). We measured total food intake in a new group of mice (n = 8) that received a single dispense of the HFD containing 50% of their daily need, once each day for 8 days, along with ad libitum chow (this feeding paradigm was identical to the 50% supplement group in Figure 3). For the following 8 days, mice continued to receive the same total amount of the HFD, but the HFD was divided into six portions that dropped once every 4 hours. Mice ate slightly more when the HFD was dispensed gradually (Figure 5D-5E; average difference: 0.5 kcal/d; P < 0.05) but not approaching ad libitum levels (Figure 4A). While we may have identified a mechanism by which food timing affects total caloric intake, it is important to note that the effect size was small, accounting for approximately 5% of daily caloric need. On an ad libitum HFD, mice overate each day by approximately 50% to 100% in the first week. We conclude that timing of the dispensing did not account for the lack of increase in total caloric intake following limited exposure to an HFD.

Finally, we predicted that mice would overconsume an HFD, but not low-fat chow, at a time when they would not normally be hungry. We gave mice access to an ad libitum HFD or chow for 30 minutes at the beginning of their light cycle (new mice, n = 32, randomized crossover design). As predicted, mice consumed approximately 50% of their daily caloric need from the HFD in this period but only 9% from chow (Figure 5E; two-tailed paired t test, P < 0.0001).

Discussion

The obesity epidemic observed in industrialized nations has been attributed in part to the wide availability of highly palatable, calorically dense foods (4,6). Foods that are high in fat are often

Figure 2 HFD leads to overeating. Mice were fed diets with varying percentages of fat derived from either (A) lard or (B) vegetable shortening for 3 days. Average daily kilocalories consumed per mouse per diet are plotted with circles for individual mice. *P < 0.05 on Sidak’s multiple comparison test. [Color figure can be viewed at wileyonlinelibrary.com]
overconsumed and contribute to weight gain in both animals and humans, but it is unclear how these foods impact the homeostatic regulation of daily caloric intake. Here, we used mice to address how an HFD impacts short-term hyperphagia. We demonstrated that mice did not overeat or gain weight, even when up to 90% of their normal daily caloric need came from the HFD. Yet the same mice overate and gained weight when given ad libitum HFD access. This suggests that calories from HFDs adequately engage the mechanisms regulating total daily caloric intake, leading us to speculate that hedonic hunger may be the primary contributor to overconsumption of HFDs in mice.

We first asked whether weight gained on an HFD was associated with increases in energy consumption or decreases in energy expenditure. After 9 weeks of an ad libitum HFD, body weight was positively correlated with food intake but also positively correlated with energy expenditure. This increase in energy expenditure in the heaviest mice may be due to HFD-induced thermogenesis (24,25). Human studies have also shown that people with obesity have higher absolute levels of energy expenditure than lean individuals (23,26,27), but their energy expenditure tends to be lower when adjusted for body weight or fat-free mass (26,27). Many of these studies measuring energy expenditure have involved cross-sectional comparisons of lean individuals and individuals with obesity who were presumably in energy balance. Yet the mice that gained weight in our study had not reached energy balance and may therefore have increased energy expenditure to compensate for their positive energy balance. Regardless of this distinction, we concluded that HFD-induced weight gain was associated with increases in energy intake but not decreases in energy expenditure.

Next, we tested whether the source of fat affected total daily caloric intake. Although the effects of specific fats on health are controversial...
unsaturated fats are generally more beneficial for health than saturated fats (29,30). To determine whether the fat source affected daily caloric intake, we provided mice with ad libitum diets with added fat from lard (46% of the fat being saturated fat and 50% unsaturated fat) or vegetable shortening (29% of the fat being saturated fat and 65% unsaturated fat). We found no significant effect of the fat source on total daily caloric intake, but mice overate both diets with higher total fat. This suggests that HFD overeating may be due to similar mechanisms in different fat types, although our examination of this subject here was limited to two sources of fat. In addition, we measured only daily calorie intake and cannot draw conclusions about other health-related outcomes from these fats. Compatible with our results, a review of 13 studies (31) demonstrated that prolonged HFD feeding increased body weight, with similar effects observed with
animal- and plant-based fats (diets ranged between 40% and 60% fat from lard, milk fat, coconut fat, olive oil, or safflower oil for 20-300 days in rats and mice). In contrast, a study (32) showed that caloric intake, weight, and fat gains were greater with 14 weeks of hard-based HFD (60% fat from lard), relative to vegetable-based HFD (60% fat from vegetable shortening) in rats. Similarly, another study (33) showed that female rats who were given ad libitum access to a diet high in saturated fats (67% fat from butter or lard) for 50 days failed to adjust their intake based on energy density, while rats with a diet high in unsaturated fat (67% fat from canola oil) did. As humans consume a wide variety of fats, further investigation of the effect of specific fat sources on energy intake and expenditure is needed.

The mechanism for overeating of HFDs and its subsequent weight gain likely involves a combination of multiple feedback mechanisms, including homeostatic, hedonic (palatability), and cognitive feedback (34). Here, administration of HFD supplements consisting of up to 90% of daily caloric requirements did not lead to increased total daily caloric intake. Additionally, the timing of HFD delivery (single or multiple dispenses) did not alter this finding. Importantly, these same mice overate and gained weight when given ad libitum access to an HFD. We concluded that HFDs appropriately engaged the mechanisms underlying homeostatic regulation of total daily caloric intake, and that overeating on an ad libitum HFD occurred despite such mechanisms. In a direct test of this conclusion, we found that mice ate approximately 50% of their daily caloric needs when given access to an HFD for 30 minutes during their light phase, a time when they normally do not consume large amounts of food. In contrast, they consumed only a minimal quantity of chow (9% of their caloric needs) under the same conditions. Although rodents can alter their circadian rhythm in anticipation of palatable foods (35), we do not think this impacted our results because we were not assessing food intake at specific time points. Additionally, the mice in this experiment could not have been expecting the HFD because we used a new cohort of mice with limited exposure to the HFD. Our findings relate to those reported by Hurley and colleagues who used a two-meal paradigm to decipher the homeostatic and hedonic drives of food intake (36). In this study, fasted rats were given access to standard chow for 2 hours, followed by 15 minutes of chow or a Western diet (17% protein, 43% carbohydrate, 41% fat; 4.7 kcal/g). Rats doubled their caloric intake of the Western diet over 2 weeks but did not increase their intake of chow.

Our findings may support weight-management strategies that limit access to high-fat foods at specific times of the day. Temporarily restricting access to a 45% and 60% HFD (fat from lard) for 8 hours per day protected mice against obesity over 4 and 12 weeks (37,38) and even led to weight loss in obese mice (38). Similarly, 12-hour restriction of an HFD inhibited the development of obesity in mice for at least 17 weeks (39). A study in humans also showed that reducing eating duration (from >14 hours to 10-11 hours per day) reduced body weight and increased self-reported energy levels in overweight individuals (40). However, more empirical tests are needed to understand the extent of time-restricted weight-loss interventions.

One reason animals overeat HFDs may be because of their palatability; HFDs are more palatable than low-fat diets and are spontaneously overconsumed by animals (41). Palatable foods activate brain reward circuits, including the ventral tegmental area, nucleus accumbens, amygdalar complex, and prefrontal cortex (42,43). Activation of these regions may drive consumption of food beyond homeostatic needs, termed “hedonic hunger.” Hedonic hunger has been increasingly recognized for its contribution to positive energy balance and development of obesity (44). However, assessing the relative contribution of hedonic pathways to overconsumption of an HFD is difficult because the behavioral and neurobiological alterations depend on dietary fat content, type of fat, and inclusion of other dietary components (45). Furthermore, hedonic and homeostatic mechanisms are not exclusive of each other; eating activates areas of the brain known to regulate food intake as well as areas regulating reward and motivation processing (43). However, hedonic mechanisms can override regulatory mechanisms and cause overconsumption, such as when one eats a high-calorie dessert after a satiating meal.

We conclude that HFDs engage mechanisms that regulate total daily caloric intake, yet if provided ad libitum, animals overconsume HFDs despite these mechanisms. This supports the case for HFD-induced overeating and weight gain being driven by hedonic, and not homeostatic, hunger in mice. However, feedback control mechanisms related to energy balance are complex and interactive, and developing interventions to prevent excess energy intake or achieve weight loss in humans will require further understanding of these mechanisms.

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