Timing of Feeding Behavior in HFHS Rats

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Introduction

Obesity is a global public health concern that has prompted research aimed at understanding the underlying causes and treatment of the disease. Apovian (2016) defined obesity as having a body mass index (BMI) of 30kg/m^2 or higher and found that obese individuals are more susceptible to many comorbid conditions. An exact cause for obesity has not yet been determined, as many complex biological and behavioral relationships may impact the etiology (Skelton *et al.*, 2011).

Dietary habits are thought to influence the development of obesity, and previous research has attempted to replicate human dietary habits in animals to develop an applicable obesity model. Diet-induced rat models are common, as they mimic the hormonal, neurological, and endothelial components of human obesity well (Luts & Woods, 2012). When La Fleur *et al.* (2014) investigated an obesity model describing meal patterns, food intake, and weight gain in male Wistar rats, they found that rats with a HF (high fat) diet initially consumed more, possibly due to weaker feedback to suppress ongoing eating, but compensated with a decrease in subsequent meal frequency. When administered a HS (high sucrose) diet, in liquid form, the same rats showed an increased frequency of meals, with a smaller meal size. When the two scenarios were conducted together in an fcHFHS diet (free choice; ad libitum high sugar and water; high fat or standard chow), the rats ate similar meal sizes to rats on standard diet (water and chow) but also ate more frequently. In contrast, when conducted in a ncHFHS diet (restricted; ad libitum water; pellet of sugar, fat, and standard chow), the rats showed similar feeding patterns to the HF rats. These results suggested that liquid sugar may increase meal frequency, while the HF and ncHFHS diets, which consist of high fat foods, do not. Additionally, they observed that HFHS rats had significantly higher weight gain and plasma leptin

concentrations. Another study on Sprague Dawley Rats used a HF cafeteria diet, mimicking western diets, and showed similar eating patterns as the HF diet from la Fleur *et al.* (2014) as well as an increase in weight gain (Martire *et al.*, 2013). Together, the results from rat models demonstrate how high fat and high sugar diets can induce obesity. Moreover, the results could potentially apply to obesity in humans, described by overconsumption of saturated fat and sugar-based beverages (la Fleur *et al*., 2014).

Diet-induced rat models are advantageous because they have well-defined circadian rhythms. Potter *et al.* (2012) explained that the circadian rhythm primes animals to specific feeding times that align with other biological mechanisms and the light/dark phase of their internal clocks. Circadian rhythms are not rigid and can be altered by outside factors such as diet. For example, Pendergast *et al.* (2013) observed an immediate change in eating pattern when a HF diet was introduced. They tested rats during a one-week period and provided ad-libitum regular food for 3 days before switching to a 45% HF diet for the next 3 days. When on a standard diet, most eating behavior occurred during the dark phase with several eating bouts during the light phase. Eating behavior changed promptly after switching rats to a HF diet, resulting in a more even distribution across light and dark phases, while still maintaining a rhythmic eating cycle of 30% food intake during the light phase and 70% during the dark phase. Another study by Sun *et al.* (2019) observed the effects of obesity on circadian rhythm using a HFD (diet)-induced rat model to assess the overall metabolic changes from HFD-induced obesity. The rats fed a HFD gained significantly more weight (31.66 ± 2.04 g vs. 24.70 ± 1.55 g) than those on a normal diet. Rats fed a HFD also had significantly higher serum glucose and cholesterol levels than the control group, yet they did not eat more than the controls. The HFD appeared to alter the amount and timing of hormones released, causing weight gain in the rats

(Sun et al., 2019). Diet appears to simultaneously influence weight gain and circadian food consumption in rats.

In an effort to study obesity, a team of scientists from W&L, led by Dr. Blythe, designed and tested a diet-induced rat model. Their experiment assigned male Sprague Dawley rats to either a control diet with ad-libitum access to water and standard rat chow (3.1 kcal/g, 16% calories from fat) or a HFHS diet with ad-libitum access to water, 30% sucrose solution, and high fat chow (5.24 kcal/g, 60% calories derived from fat). The high fat chow contained over 6 times more fat and 57.4% more calories than the standard chow. From data supplied by Dr. Blythe (n = 11), our team investigated whether the HFHS diet affected the timing of drinking and food consumption. From previous research, we expected HFHS rats to have a more even distribution of feeding and drinking across the light and dark phases, while those in the control group would maintain typical habits, feeding and drinking mainly during the dark phase. The results of this analysis can potentially aid in a better understanding of how diet-induced changes contribute to obesity.

Results

We first analyzed weight differences between the conditions. The HFHS rats weighed 28.44 grams more than the control rats (p = 0.0624). To conduct our analyses on the timing of eating behavior, we parsed out feeding and drinking data during the day and night. Rats are nocturnal animals, meaning that their subjective day and night mirrors that of diurnal animals (i.e. humans). Therefore, the rats' subjective night falls during the light phase while their subjective day falls during the dark phase. In our analyses, the hours from 8:00 A.M. to 7:59 P.M. were designated as night, while the hours from 8:00 P.M. to 7:59 A.M. were designated as

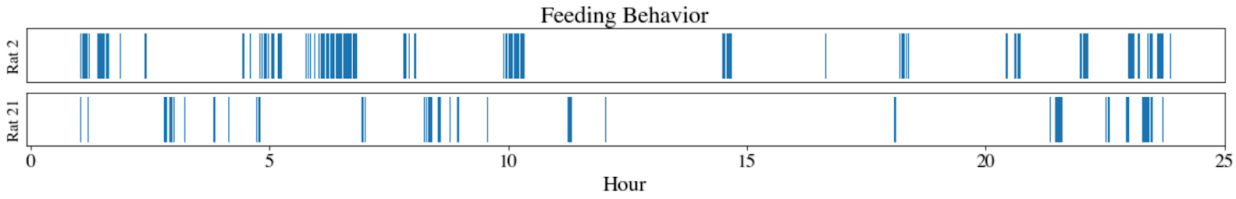
day. Drinking and eating behaviors were evaluated by two methods: frequency and duration. For duration, we found the total number of seconds each rat spent feeding or drinking. For frequency, we found the total number of discrete events in which the rat initiated a feeding or drinking behavior. The durations and frequencies of drinking and feeding were compared between HFHS and control rats at night and during the day.

For eating behavior, the control rats ate for an average of 1957 more seconds ($p = 0.0064$) and 20 more times than the HFHS rats during the day ($p = 0.0204$) . We discovered similar results for nighttime eating behaviors. At night, the control rats ate an average of 970 seconds longer ($p = 0.0244$) and an average of 11.86 more times than the HFHS rats ($p = 0.0423$). Feeding is more frequent and longer for HFHS rats at all points of the day (See Figure 1). The data for the other rats in the control and HFHS conditions tend to show similar trends (See Appendix A).

Figure 1.

Feeding Behavior in Control Rat 2 and HFHS Rat 21 across a 24-hour day, starting at 12:00AM, and concluding at

11:59PM.



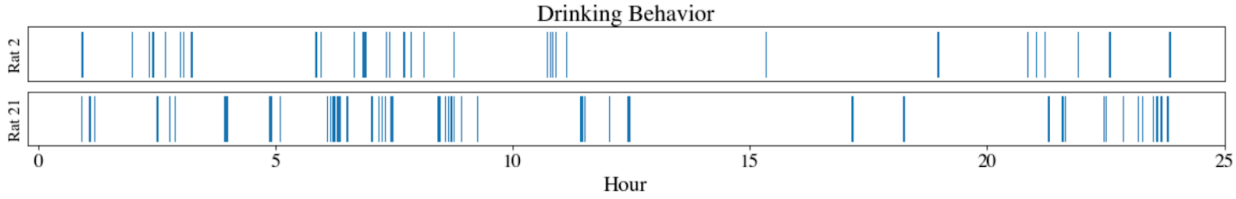
Note. Rat 2 was fed ad-libitum control chow while Rat 21 was fed ad-libitum HFHS chow. Each vertical bar represents the time frame from the beginning of a feeding behavior to the end of a feeding behavior.

For daytime drinking duration, there were no significant differences between the groups. The HFHS rats drank, on average, for 190 seconds longer ($p = 0.1725$) and 0.63 more times than the control rats during the day ($p = 0.3781$). At night, there was a significant difference between drinking duration. HFHS rats drank 215 seconds longer than control rats ($p = 0.0247$). However, frequency results were not significantly different. Control rats drank only 1 more time, on average, during the night compared to the HFHS rats ($p = 0.4352$). Drinking is more frequent at nighttime, from 8:00 AM to 7:59PM, in HFHS rats than control rats (See Figure 2). The drinking behaviors for the rest of the control and HFHS rats tend to have similar patterns (See Appendix B).

Figure 2.

Drinking Behavior in Control Rat 2 and HFHS Rat 21 across a 24-hour day, starting at 12:00AM and concluding at 11:59

PM.



Note. Rat 2 was provided ad-libitum access to water while Rat 21 was provided ad-libitum access to water and 30% sucrose solution. Each vertical bar represents the time frame from the beginning of a drinking behavior to the end of a drinking behavior.

Discussion

Our results show that rats in the HFHS treatment condition had significantly shorter feeding durations and decreased feeding frequency compared to the control condition for both day and nighttime feeding behaviors. Drinking behavior, however, only showed significant differences in nighttime drinking duration. HFHS rats had a significantly longer duration of drinking at night, while all other findings were insignificant.

Our results demonstrate how HFHS diets can change the amount of feeding, but not the timing of feeding. The decrease in feeding frequency and duration seen in the HFHS rats aligns with findings from rats given a HF and ncHFHS diet in la Fleur *et al.* (2014). Contrary to their research with fcHFHS rats, our rats on the HFHS diet spent less time eating and ate less frequently at all time points. Likewise, in contrast to findings by Pendergast *et al.* (2013), the HFHS diet in this study did not induce longer or more frequent nighttime eating for the rats or change the timing of feeding. Consequently, our findings disagree with the proposed theory that liquid sugar increases meal frequency (la Fleur *et al.*, 2014). Rather, our results suggest HFHS diet decreases feeding duration and frequency during the day and night, possibly due to the increased caloric content of the HFHS diet. Consuming a diet higher in calories, like that of the HFHS diet, may lead to increased satiation, and a subsequent decrease in hunger throughout the day.

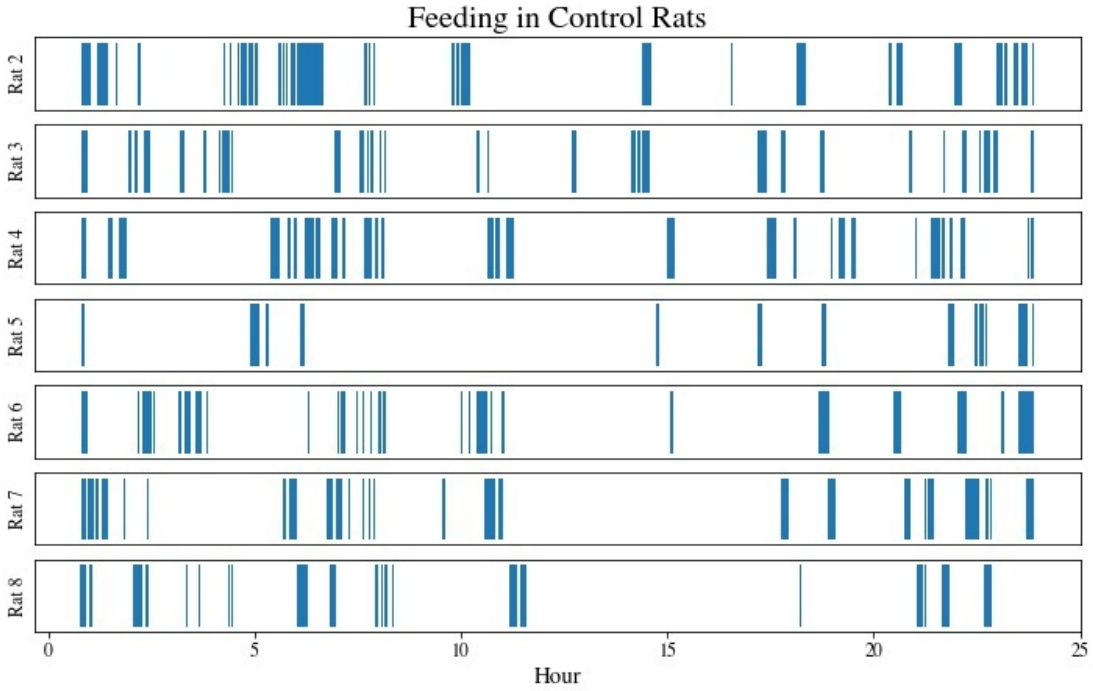
In regard to the timing of drinking, our data appears to align more closely with what Pendergast *et al.* (2013) discovered. HFHS rats drank for significantly longer at night compared to the control rats. This suggests that the addition of the sucrose may have changed these rats' typical circadian drinking patterns. All other drinking behaviors did not change significantly, suggesting that further research is necessary to establish a relationship between high-sucrose

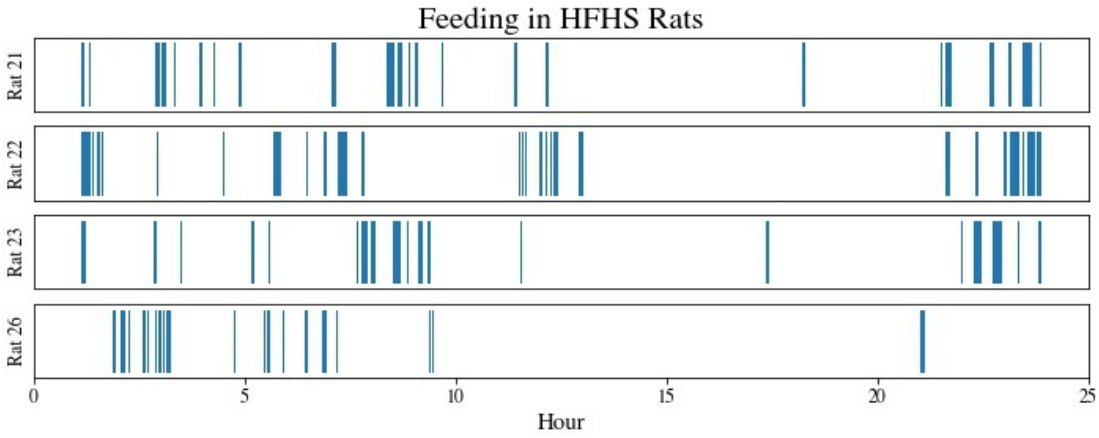
drinks and timing of drinking. It is important to note that we only compared the variation between day and night hours; future hour-by-hour analysis could uncover differences in more specific intervals associated with a HFHS diet.

The current findings that HFHS diets led to less feeding behavior in rats may be similar to those found by Sun *et al.* (2019), in which rats on a HF diet did not eat more, but still gained more weight than those on a control diet. Therefore, eating dense, high fat or high sugar food may appear to be a good idea, in that it decreases the amount you eat, but this may not really be the case. According to Sun *et al.* (2019), weight gain was due to hormone alterations caused by the HF diet. Future research should continue to address hormone levels and potential differences in the timing of hormone release, in addition to the timing of feeding and drinking.

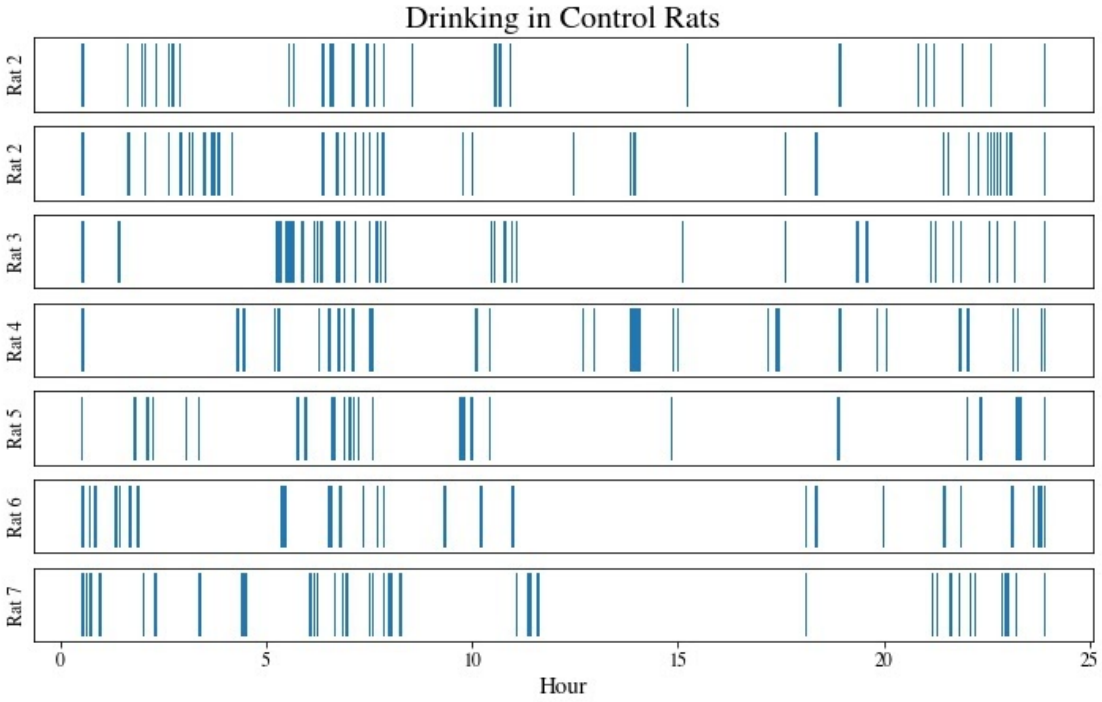
Humans are often inclined to focus on the type of food that they consume, as opposed to the timing of eating. Our results, along with previous research, indicate that consumption of a diet high in fat and sugar does not only change the number of calories consumed but also normal circadian patterns of caloric consumption, particularly the duration of nighttime drinking behavior. Overall, to prevent unwanted weight-gain, we suggest that humans should avoid food and drinks with high fat and sugar content. Unfortunately, this is difficult to do within a Western Diet, in which the easiest and tastiest things to eat are often those high in fat and sugar. Kahleova *et al.* (2017) studied feeding behaviors in humans and found that individuals who ate less frequently, did not snack, and ate their largest meal in the morning reported lower BMIs. In accordance with these results and results from the rat models, we suggest that consumption of all calories, regardless of the content, should occur during normal waking hours, as feeding and drinking outside normal circadian patterns may be connected to increased weight gain and risk of obesity.

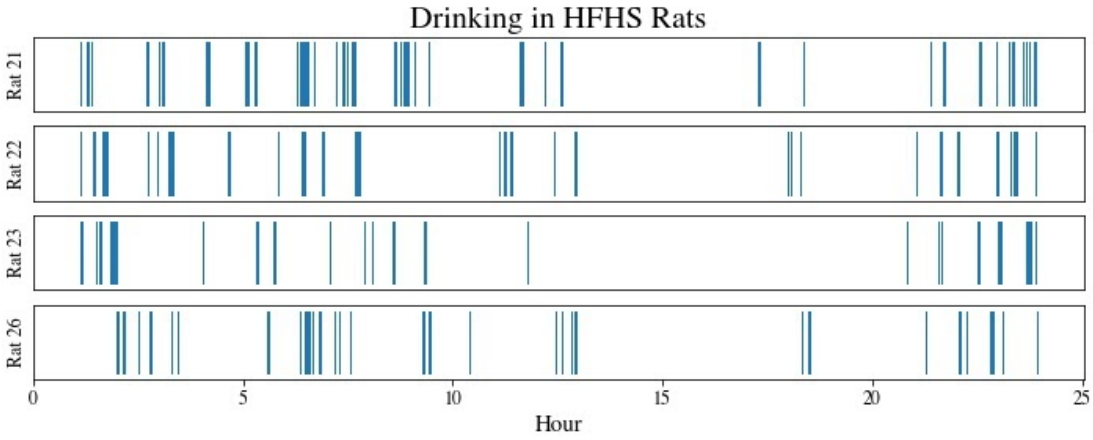
Appendix A: Feeding Behavior of All Rats





Appendix B: Drinking Behavior of All Rats





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