Alyssa Santiago

Dr. Weber

BHP Capstone

05 May 2021

Food Anticipatory Activity in Female Mice With and Without Wheels

Abstract

There is a trend in research to use male mice for research. Little is known about female mice food anticipatory activity (FAA), but what is known is that female mice have less FAA, while males experience enhanced FAA with the presence of a wheel. The study sought to determine whether female mice FAA response is enhanced with a wheel. Male and female C57BL/6 mice were used and placed in cages with a functional wheel or a locked wheel. Motion and wheel data were collected, and a 4-hour food restriction was placed on the mice. Ultimately, males and females showed no difference in FAA, and the presence of the wheel did not enhance either sex's FAA. The insignificant differences between the groups may be due to the mice being calorically restricted.

Introduction

There is a long-standing trend in research to use only male animal models in experiments for fear the female hormone cycle would make data too variable to draw conclusions from. In circadian biology, the bias against female mice is strong and prevalent, despite research showing female mice to not be significantly variable in data (5). However, female mice have important differences in circadian biology that need to be studied, such as having shorter circadian periods in certain species, and women typically waking up earlier than men (5, 6). There is limited

research in food anticipatory activity in female mice, which is the activity that occurs before a scheduled feeding time when mice are anticipating their meal. Most research indicates that females have less robust FAA than male mice, yet these studies are conducted in the presence of a running wheel, which is shown to increase the robustness of FAA response in male mice, but was not studied in female mice (6,2,5). Evidence suggests that long days increase the robustness of the FAA in male mice with wheels (2,9). It could be possible that female mice do not experience an increase in FAA with the presence of a wheel.

Moreover, different diets affect mice differently depending on sex. Female mice are protected against obesity when fed on high-fat diet, compared to males who become obese (3). The amplitude of locomotor rhythms in female mice is also not affected by diet, whereas male mice experience decreased locomotor amplitude when on a high-fat diet (7). Continuing, high fat has been shown to improve FAA robustness (4), although other research suggests a high-fat diet dampens FAA activity in 4-week old mice (6). In previous experiments done in Dr. Weber's lab, there was no significant difference in the robustness of FAA between C57BL/6 mice on high-fat and low-fat diets. Therefore, it is important to also look at the role of diet on how it can affect FAA between males and females. The goal of this study is to grow an understanding of female mice circadian biology by determining whether or not female mice FAA is altered by the presence of the wheel during different diets.

Methods

In order to test the effects of wheel running on female mice FAA, mice were split into 4 groups of n=5. One included females in their own cages with functional exercise wheels and another group included females with locked wheels. The same was done for males. Each shelf

housed one mouse from each group to avoid loss of data of an entire group in the unfortunate event the light bulb on one shelf ceases to function. Relatively young C57BL/6J mice of approximately 6-10 weeks old were used to compare to the University of Texas Southwestern study (2), as evidence suggests in rat models that age decreases the robustness of FAA (10). Wheel running data was collected for those housed with functional wheels, while infrared motion sensors were fixed above all cages to determine if mice participate in activity outside of the wheel running, as well as to compare the activity of mice with wheels to mice without. Body weights were collected throughout the experiment to determine the effects of restricted feeding on food intake and as a measure of health.

Lights were on for 16 hours and off for 8 hrs each day (LD 16:8). Mice were entrained to the light cycle on a high-fat diet in their respective cages before food restriction. However, no FAA was apparent in the actograms of any mice during the restriction, so the high-fat lab diet was replaced with low-fat chow later on in the experiment. Food was gradually restricted to mitigate the initial loss of body weight and to give mice time to get used to the new restricted feeding schedule. Food normally available for 24 hours was restricted to 12 hours for 2 days, then 10 hours, 8 hours, 6 hours, and 4 hours, in increments of 2 days, until a 4-hour restriction was reached. Food was placed at ZT7 during the duration and gradually taken off earlier and earlier until the food was taken off at ZT11. Food was placed in a dish at the bottom of each cage, as prior research has shown mice to become lethargic and lose weight if food is placed on top of cages (8). After accumulating to high-fat diet restriction, ad-lib feeding took place for 3 days to determine if FAA persists. Mice were then re-entrained to the restricted feeding schedule, and food was replaced with low-fat chow. The restriction was increased to 5 hours from 4 hours

to help mitigate weight loss. 1 male mouse with a locked wheel was pulled from the experiment due to weight loss. After FAA became apparent, a 3-day ab-lib vacation was given to mice. Then, lights off and lights on were delayed to six hours.

At the end of the experiment, activity was measured and compared using both wheel-running data and motion sensor data to determine any sex differences between male and female activity under restricted feeding and change in diet. Statistics were performed using SPSS.

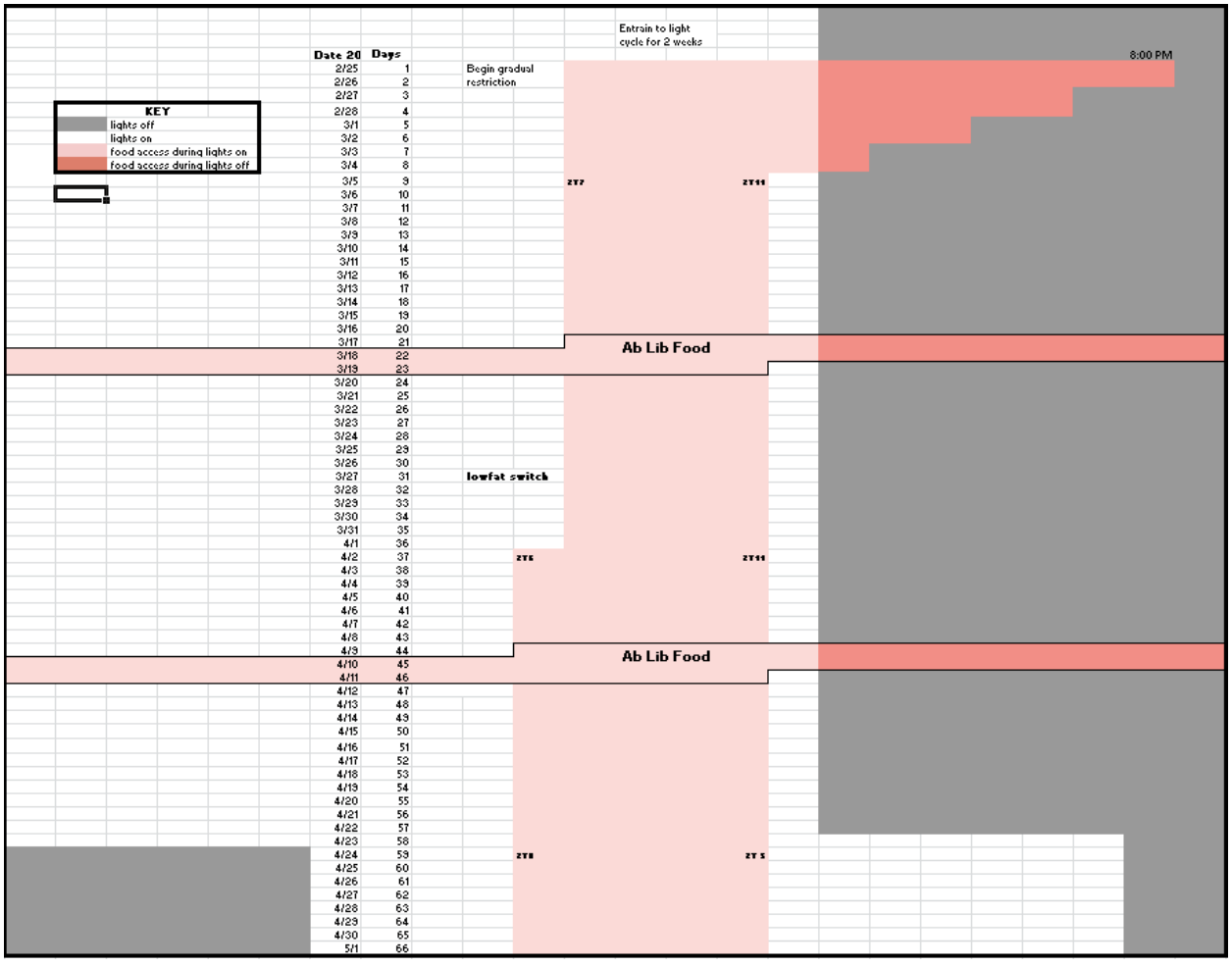

Figure 1 Experimental schematic of restricted feeding schedule and day-light cycle. Based on a 16L:8D day.

Results

Before restricted feeding began, data was collected to determine how much HFD mice were eating. Females with free access to wheels ate 2.36g (SE=0.19), females with locked wheels ate 2.36g (SE=0.04), males with free wheels ate 2.88g (SE= 0.24) and males with locked wheels ate 2.58 (SE=0.11).

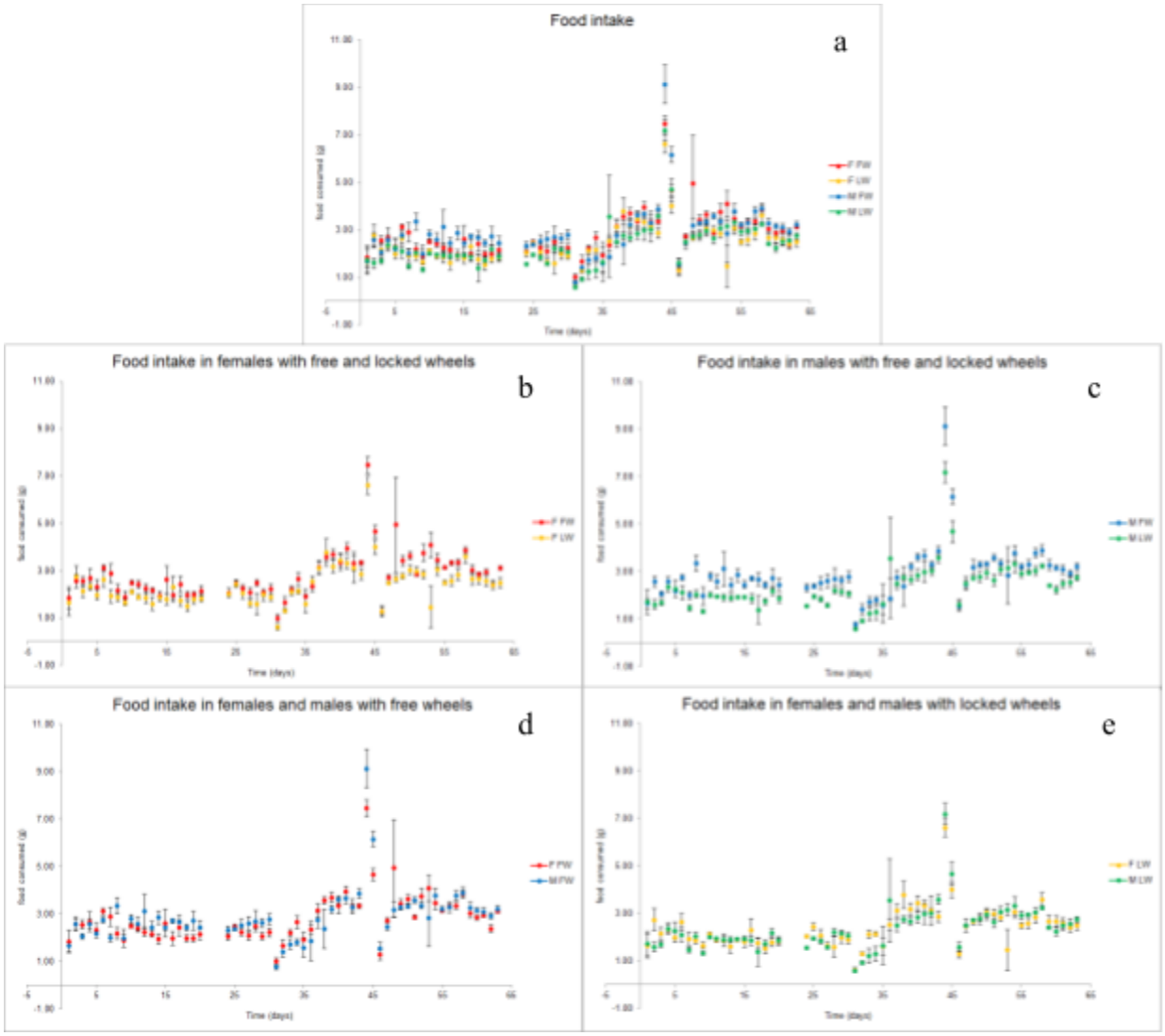

Figure 2 Food intake by weight during restrictive feeding and ab-lib for high and low-fat diet. Days 1-8 were gradual food restrictions on HFD, days 9-20 were HFD. Day 24-30 was HFD post ab lib vacation, days 31-43 was LFD, and days 44-46 was ab lib LFD.

The presence of a functional wheel in female mice only increased food intake during HFD before ab lib vacation, but not after ($p<0.001$). In male mice, the presence of a functional wheel increased food intake during HFD before and after ab lib vacation ($p<0.001$). Food intake during ab lib LFD was significantly higher than all previous treatments in each group ($p<0.001$).

Females with locked wheels were the only group to experience a difference in food intake between HFD and LFD ($p<0.001$). Sex and wheel access was shown to have significant impacts on food intake ($p<0.001$).

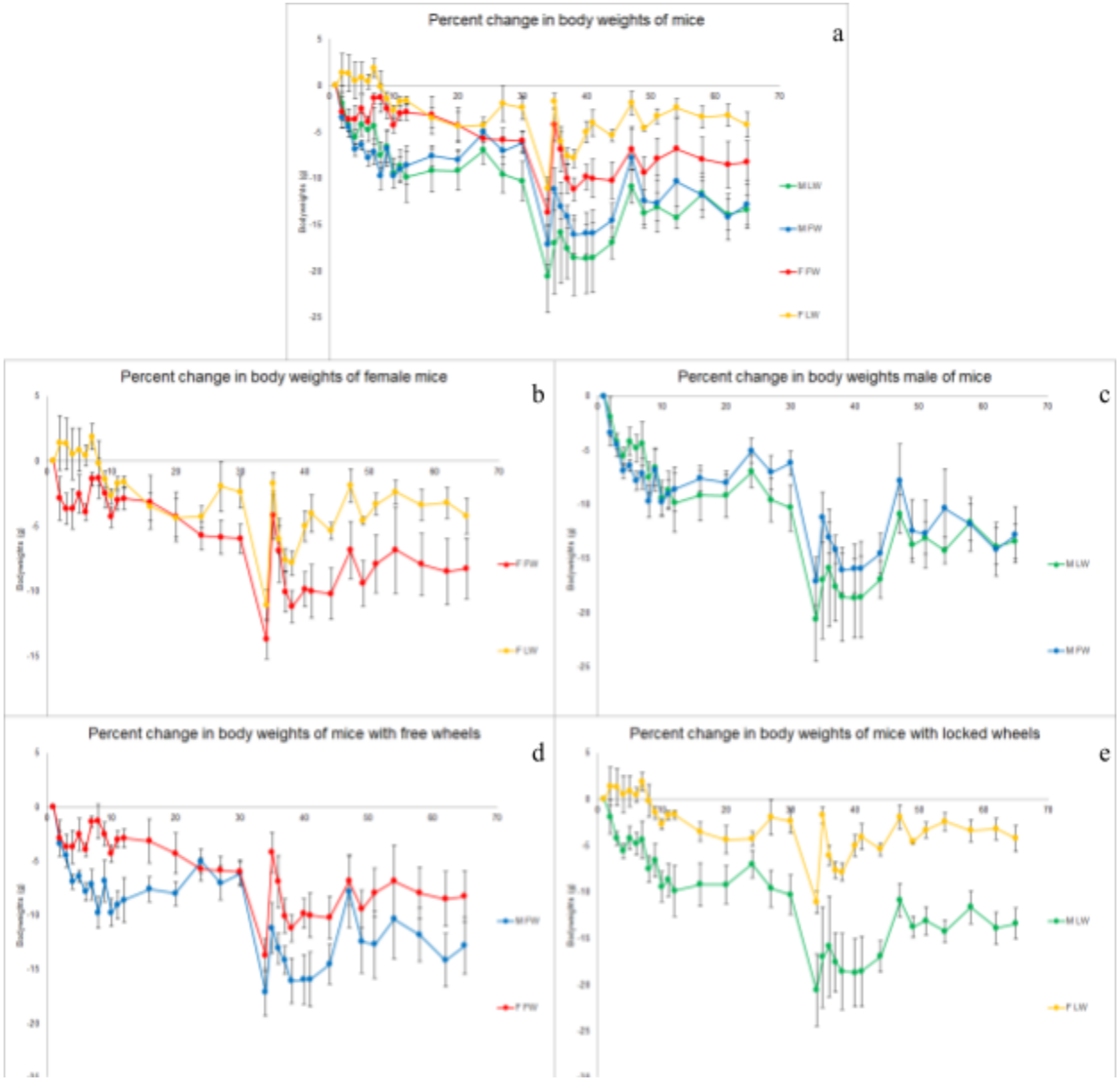

Figure 3 Percent change in body weight of mice throughout restricted feeding schedule and ab-lib vacations. Days 1-8 were gradual food restrictions on HFD, days 9-20 were HFD. Day 24-30 was HFD post ab lib vacation, and days 31-43 was LFD. On day 34, an extra pellet was left in each cage after food was taken off to mitigate weight loss.

Gender and wheel access had a significant impact on change in body weight ($p<0.001$). In males with free wheels and males with locked wheels, LFD had significantly decreased body

weight compared to all other treatments ($p<0.001$). In females with locked wheels, the gradual restriction had a significant increase in body weight compared to all groups, while LFD had significantly more negative changes in body weight between HFD ($p<0.001$). In females with free wheels, LFD had significantly more negative changes to body weight compared to gradual restriction and HFD ($p<0.001$). Wheel access was only shown to have a significant effect on change in body weight in female mice during gradual food restriction ($p<0.001$), but wheel access did not have a significant impact on male mice with or without free wheels in their change in body weight.

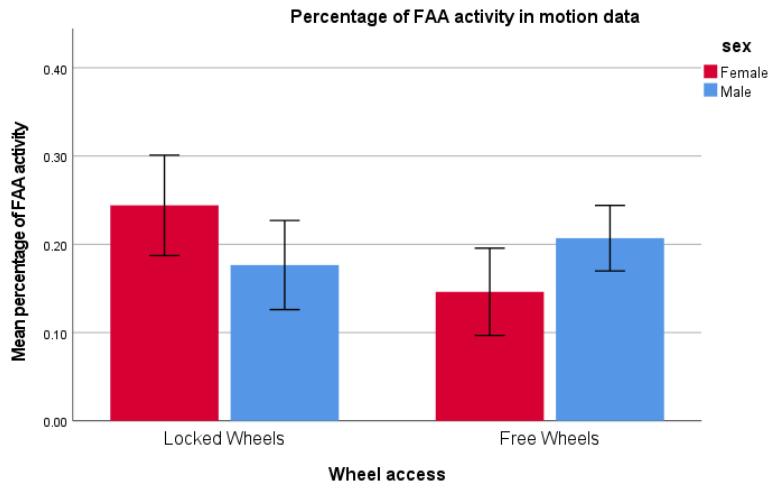

Figure 4 Percentage of motion data dedicated to FAA in female and male mice with or without free wheels (SE)

Counts were taken after LFD ab lib. There were no significant differences in sex and wheel access. Sex and wheel access had no effect on the percentage of activity making up FAA.

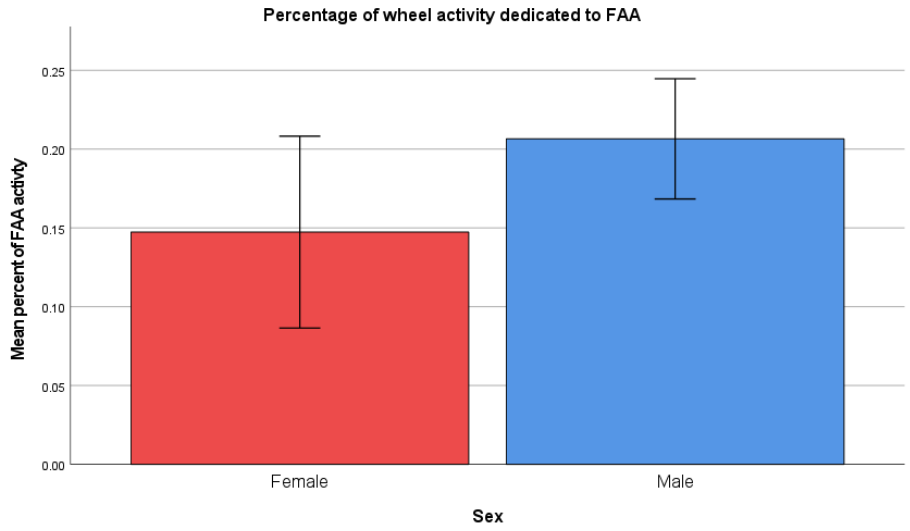

Figure 5 Percentage of wheel data dedicated to FAA in female and male mice

Wheel counts were taken in the same timeframe as motion counts. There were no significant differences in the percent of activity dedicated to FAA due to sex. Sex had no effect on wheel running dedicated to FAA.

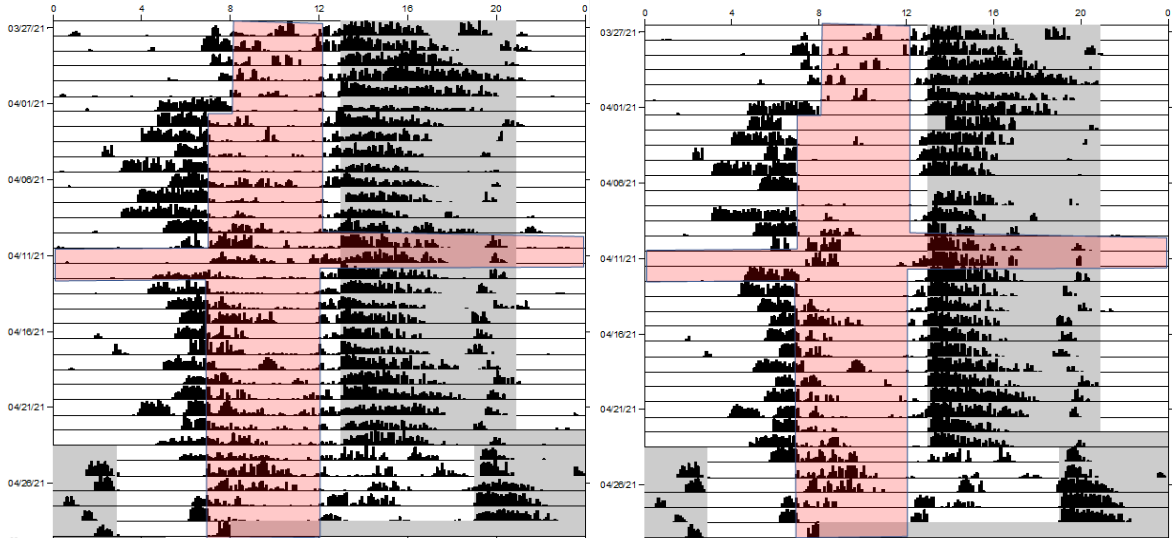

Figure 6 Example mouse actogram for motion (left) and wheel data (right). The red box is the LFD feeding schedule.

During ab lib food access, all mice experience either a decrease or disappearance of FAA. Moreover, when the lights were shifted to come on at a later time, FAA in all mice either disappeared or decreased in strength.

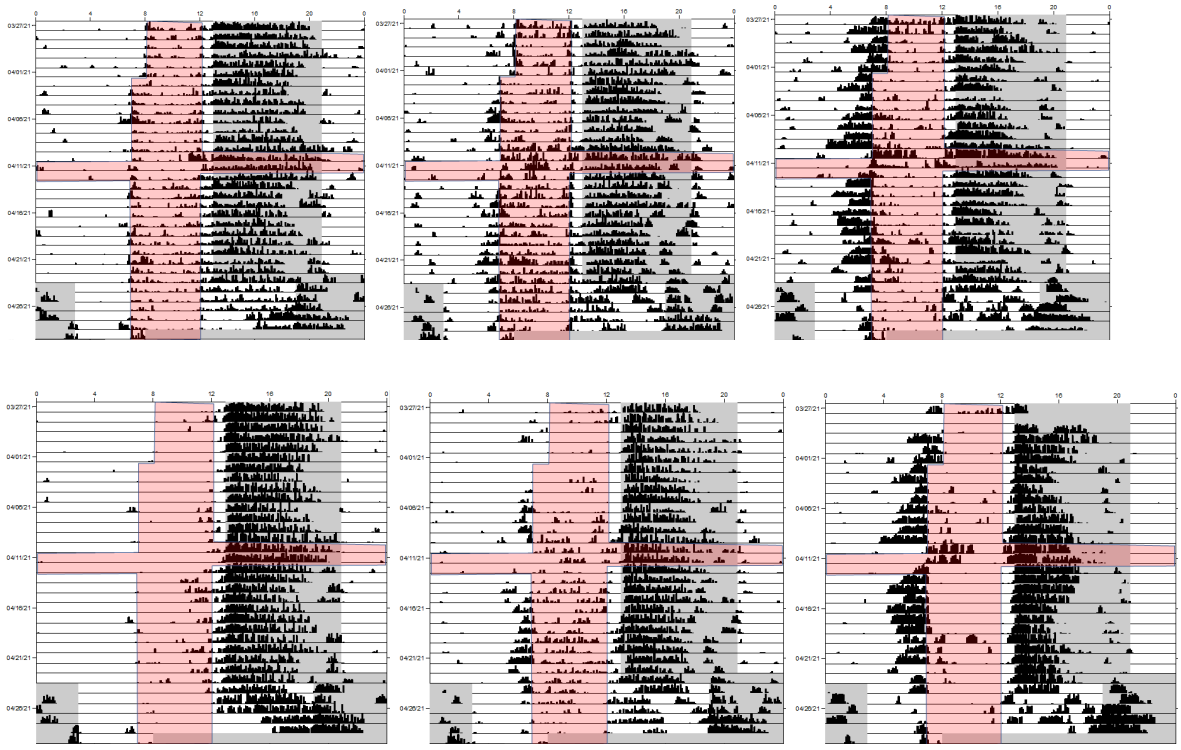

Figure 7 Mice motion (top) and wheel (bottom) actograms of 20 week, 18 week, and 17-week old females (left to right) with freewheels.

In each group, mice appeared to experience a more prominent FAA response the younger they were. Age was slightly distributed throughout each group, with the first actogram being the oldest mouse in the group to the last being the youngest.

Discussion

On HFD, mice ate relatively the same on ab lib food that they did during restriction once the restricted feeding protocol began. However, on LFD, their ab lib food intake sharply increased (Figure 2a). It is possible that on HFD, mice were not calorically restricted, yet on LFD, mice were in a calorie deficit (5, 9). In the future, food intake by weight will be converted

to calorie intake to determine whether or not mice are calorically restricted, and if mice are eating based on the weight of the food, or calorie content of the food.

In male mice, the presence of a wheel increased food intake while in female, a wheel had no significant effect on food intake (Figure 2b,2c). Previous research suggests that males typically spend more time running on wheels than females, while female behavior typically focuses on cage top climbing and food bin entry (5). It could be possible that males are burning more calories by using a wheel more frequently and therefore may need to consume more calories.

Female mice experience less extreme changes in weight than male mice (Figure 3a). Previous research has shown that female mice are more resistant to obesity, and do not gain as much weight as male mice. (3,5). It could be possible that similar to being resistant to gaining weight, female mice may also be resistant to losing it as well, resulting in a more stable weight throughout the experiment compared to their male counterparts.

There was no effect of wheel availability or sex on FAA (Figure 4, 5). It could potentially be due to the mice potentially being calorically restricted. There may be no effects of sex or wheel access on FAA when a caloric restriction is placed instead of a food restriction. Moreover, mice lose their FAA response during ab lib food access, suggesting that hunger from the caloric restriction was driving FAA (Figure 6). Mice also lose FAA response during phase delay, a phenomenon that may be investigated in the future (Figure 6). Lastly, it was discovered that mice may potentially have a weakened FAA response as they age (Figure 7). The older mice did not show prominent FAA as the younger mice, yet a much larger sample size is needed for future experiments in order to make a conclusion.

Overall, the experiment must continue for longer in order to analyze actogram data of activity to determine what exactly is happening during the hours of the day that causes a sex difference in change in body weight. It could be possible that males are more active than females, or that there is a physiological component as previously suggested (3,5).

References

1. Duncan, M. J., Smith, J. T., Narbaiza, J., Mueez, F., Bustle, L. B., Qureshi, S., Fieseler, C., & Legan, S. J. (2016). Restricting feeding to the active phase in middle-aged mice attenuates adverse metabolic effects of a high-fat diet. *Physiology & behavior*, *167*, 1–9. https://doi.org/10.1016/j.physbeh.2016.08.027

2. Flresô, D. E., Bettilyon, C. N., Jia, L., & Yamazaki, S. (2016). The Running Wheel Enhances Food Anticipatory Activity: An Exploratory Study. *Frontiers in behavioral neuroscience*, *10*, 143. https://doi.org/10.3389/fnbeh.2016.00143

3. Freire, T., Senior, A. M., Perks, R., Pulpitel, T., Clark, X., Brandon, A. E., Wahl, D., Hatchwell, L., Le Couteur, D. G., Cooney, G. J., Larance, M., Simpson, S. J., & Solon-Biet, S. M. (2020). Sex-specific metabolic responses to 6 hours of fasting during the active phase in young mice. *The Journal of physiology*, *598*(11), 2081–2092. https://doi.org/10.1113/JP278806

4. Gallardo, C. M., Gunapala, K. M., King, O. D., & Steele, A. D. (2012). Daily scheduled high fat meals moderately entrain behavioral anticipatory activity, body temperature, and hypothalamic c-Fos activation. *PloS one*, *7*(7), e41161. https://doi.org/10.1371/journal.pone.0041161

5. Krizo, J. A., & Mintz, E. M. (2015). Sex differences in behavioral circadian rhythms in laboratory rodents. *Frontiers in endocrinology*, *5*, 234. https://doi.org/10.3389/fendo.2014.00234

6. Li, Z., Wang, Y., Sun, K. K., Wang, K., Sun, Z. S., Zhao, M., & Wang, J. (2015). Sex-related difference in food-anticipatory activity of mice. *Hormones and behavior*, *70*, 38–46. https://doi.org/10.1016/j.yhbeh.2015.02.004

7. Palmisano, Brian T et al. "High-Fat Feeding Does Not Disrupt Daily Rhythms in Female Mice because of Protection by Ovarian Hormones." *Frontiers in endocrinology* vol. 8 44. 14 Mar. 2017, doi:10.3389/fendo.2017.00044

8. Pendergast, J. S., Nakamura, W., Friday, R. C., Hatanaka, F., Takumi, T., & Yamazaki, S. (2009). Robust food anticipatory activity in BMAL1-deficient mice. *PloS one*, *4*(3), e4860. https://doi.org/10.1371/journal.pone.0004860

9. Power, S. C., & Mistlberger, R. E. (2020). Food anticipatory circadian rhythms in mice entrained to long or short day photoperiods. *Physiology & behavior*, *222*, 112939. https://doi.org/10.1016/j.physbeh.2020.112939

10. Shibata, S., Minamoto, Y., Ono, M., & Watanabe, S. (1994). Age-related impairment of food anticipatory locomotor activity in rats. *Physiology & behavior*, *55*(5), 875–878. https://doi.org/10.1016/0031-9384(94)90073-6