The impacts of obesity-promoting diets on feeding quantity over time in ^{w1118} *Drosophila melanogaster*

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Abstract

High-sugar diets (HSDs) and high-fat diets (HFDs) cause pathogenic outcomes such as insulin resistance, obesity, shortened life span, cardiomyopathy, and hyperglycemia in *Drosophila melanogaster*. The HFD we utilize contains a 20% w/v coconut oil content compared to a normal 0% w/v coconut oil content, while the HSD contains 20% w/v sucrose as opposed to a normal 3% w/v sucrose. Previously, flies were found to reduce their feeding quantity on HSD relative to NM (normal medium) after 7 days of exposure. This behavior raised questions as to what extent these diets induce aversion or changes in feeding behavior, particularly in mated w^{1118} females. We found that when placed on a HSD for 7 days and then exposed to normal and HSD for 4, 8, 16, 24, and 48 hours, flies generally had increased feeding quantity on NM compared to HSD. However, flies with previous maturation on NM exhibited a semi-cyclic pattern of feeding, where there was a significant downregulation in response to HSD at 4 hours that does not exist at 8 hours, only to reemerge at 24 hours, and disappear again at 36 hours. Flies with previous maturation on HSD exhibited no such pattern, showing general reduction in feeding on HSD, but there were no statistically significant differences at each time point. When flies were placed on a HFD for 7 days and then exposed to HFD and NM for 4, 8, 16, 24, and 48 hours, they again showed an overall decrease in feeding on the HFD compared to normal diet. When flies previously exposed to NM were exposed to NM or HFD, they exhibited a cycle pattern of feeding with two distinct feeding peaks over a 48 hour period. In contrast, when flies previously exposed to HFD were exposed to NM or HFD, they exhibit only one distinct feeding peak over 48 hours. HFD pre-exposed flies did not experience the same downregulation in response to HFD feeding at around 36 hours compared to NM pre-exposed flies. However, feeding did decrease at around 48 hours in HFD pre-exposed flies. These observations pose questions regarding appetitive homeostasis, circadian rhythm, and potential disruption with HSD and HFD exposure.

Objectives

- Evaluate the effects of prior exposure to normal and high sugar media on feeding quantity on normal and high sugar media over time
- Gain further insight into the feeding decline observed in mated females exposed to high sugar diet as part of an earlier Talbert lab DGRP study

Methods

Media: Flies were reared on a standard diet consisting of 5.2% cornmeal, 5.0% yeast extract, 1.0% agar, 3.0% sucrose, 1.5% tegosept (20% w/v in 70% ethanol), 0.3% v/v propionic acid and 0.3% tetracycline (10 mg/mL in 70% ethanol). The normal diet (NM) used for the feeding quantity assay using high sugar diet (HSD) consisted of 2.6% cornmeal, 4.0% yeast extract, 0.8% agar, 3% sucrose, 1.5% tegosept and 0.3% propionic acid. HSD consisted of the same amount of ingredients as the NM, except the sucrose content was increased to 20%. The NM used for the feeding quantity assay with high fat diet (HFD) consisted of 5% yeast, 1.5% agar, 5% sucrose, 1.5% tegosept, and 0.3% propionic acid. HFD consisted of the same ingredients as the NM, but with a coconut oil content of 20%. The percentages are w/v unless otherwise noted.

Flies: The w^{1118} flies (Stock 5905) were received from the Bloomington Drosophila Stock Center (BDSC). Virgin female experimental flies were housed at an equal population density. Flies used for feeding quantity were stored for maturity 2-3 days and then mated with male siblings for 48 hours in a 3:1 ratio. Males were removed and the mated females were subjected to the normal, high fat, or high sugar diet for 7 days.

Feeding Quantity: After 7 days on exposure to experimental diets, five replicates of 5 adult female mated flies were transferred onto experimental diets containing 1% Brilliant Blue dye. (N=5, 5 per replicate, 25 flies per condition). Flies were left undisturbed to feed on the Brilliant Blue media for 4, 8, 16, 24, 36, and 48 hours at 23ºC. Before homogenization, flies were held in the -20ºC freezer at the conclusion of their feeding duration. The flies were then homogenized in 400 µL PBT using an electric pestle and the homogenate was centrifuged for 20 minutes at 13,000 RPM. The supernatant was extracted and a triplicate of 100 µL of each sample was pipetted into a 96-well plate in a randomized order. The amount of food ingested was quantified by measuring the absorbance of the supernatant extracted at 625 nm in the Biotek plate reader and interpolating the concentration of the blue dye with Brilliant Blue standards in PBT.

Results

Flies exhibited a cyclic pattern of feeding downregulation on HSD relative to normal diet after prior normal diet exposure.

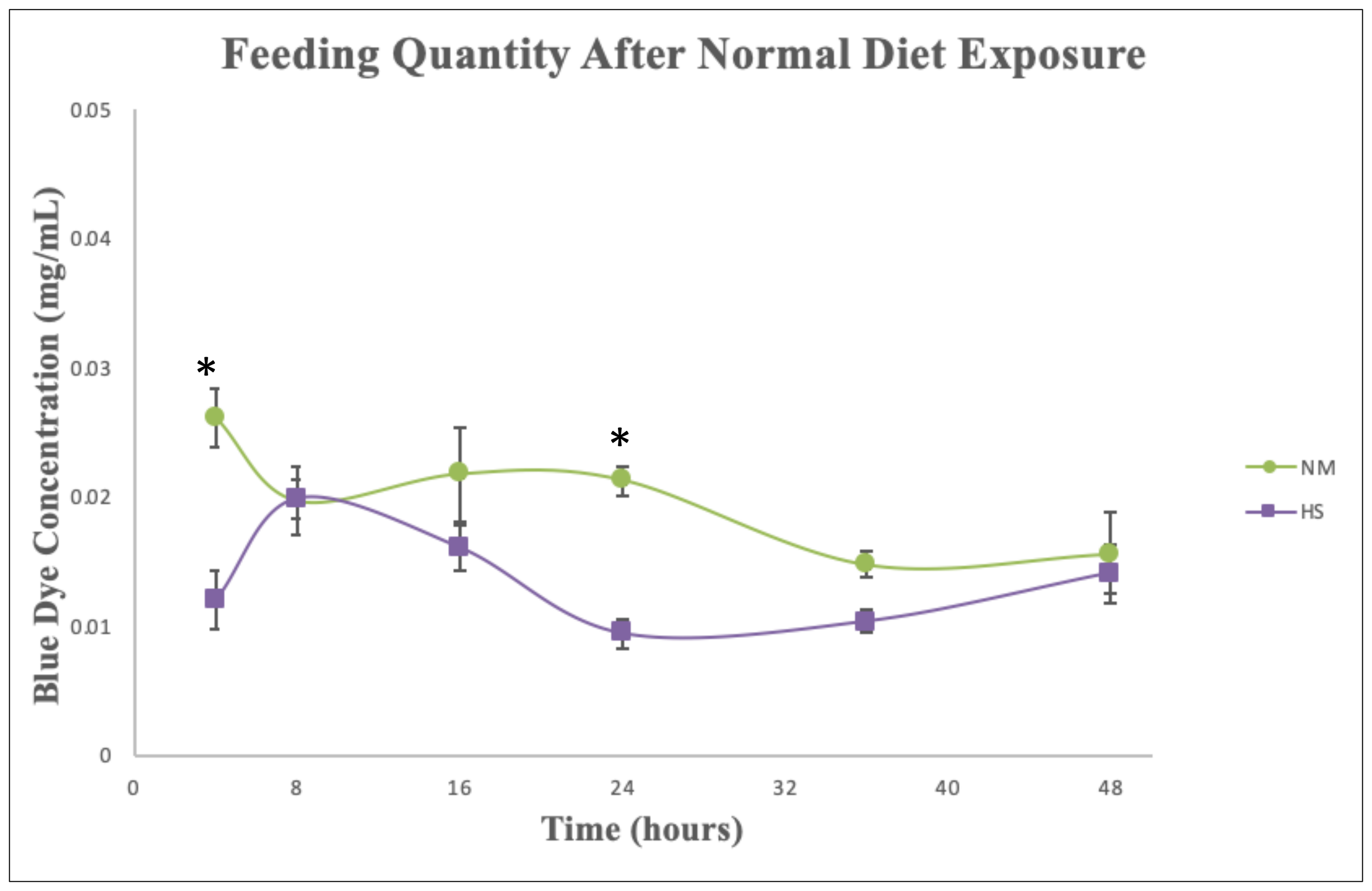

Figure 1: Effect of normal diet exposure on feeding quantity upon normal and high sugar substrates. After prior exposure to a normal diet, flies were seen to exhibit significant downregulation of high sugar feeding after 4 and 24 hours of feeding as per a Student's T test (P<.05). High sugar diet feeding was seen to increase at 8, 36, and 48 hours, such that there was no difference between feeding on normal and high sugar diets, indicating a cyclic pattern that could be related to circadian rhythm and periods of active foraging. Error bars indicate standard error of mean (SEM). Stars indicate differences in timepoints compared to high sugar feeding after previous high sugar exposure.

Flies generally decrease their feeding quantity on HSD relative to normal diet after being previously exposed to HSD. There is no cyclic pattern.

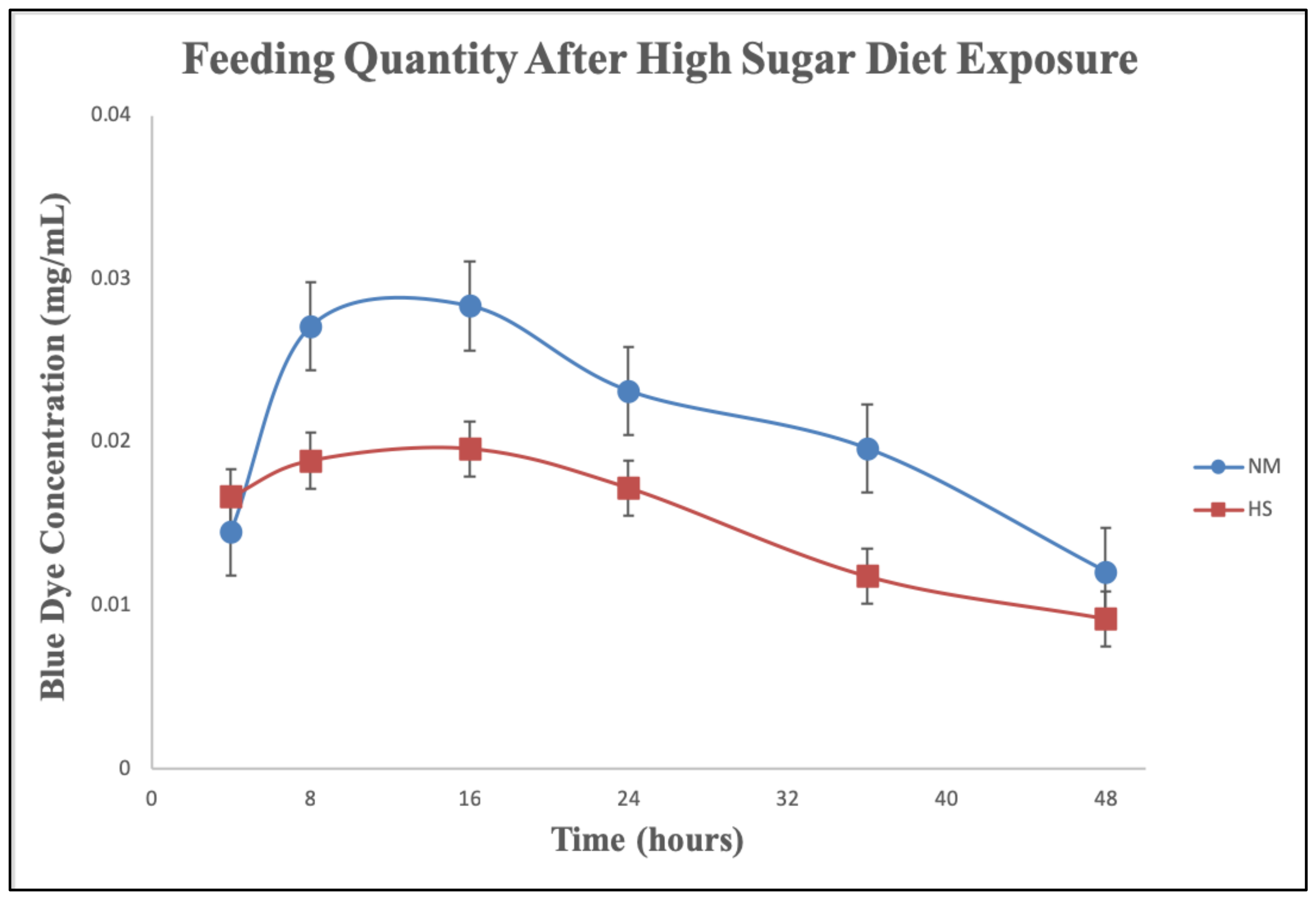

Figure 2: Effect of high sugar exposure on feeding quantity upon normal and high sugar substrates. After prior exposure to a high sugar diet, flies were seen to increase their feeding on blue normal diet compared to high sugar diet. None of these time points indicated significant differences between feeding quantity as per a Student's T test. The cyclic pattern of HSD downregulation seen in Figure 1 is absent, indicating a change in feeding behavior. Error bars indicate standard error of mean (SEM).

Results (Continued)

Flies also presented a cyclic pattern of feeding downregulation on HFD compared to normal diet after normal diet exposure.

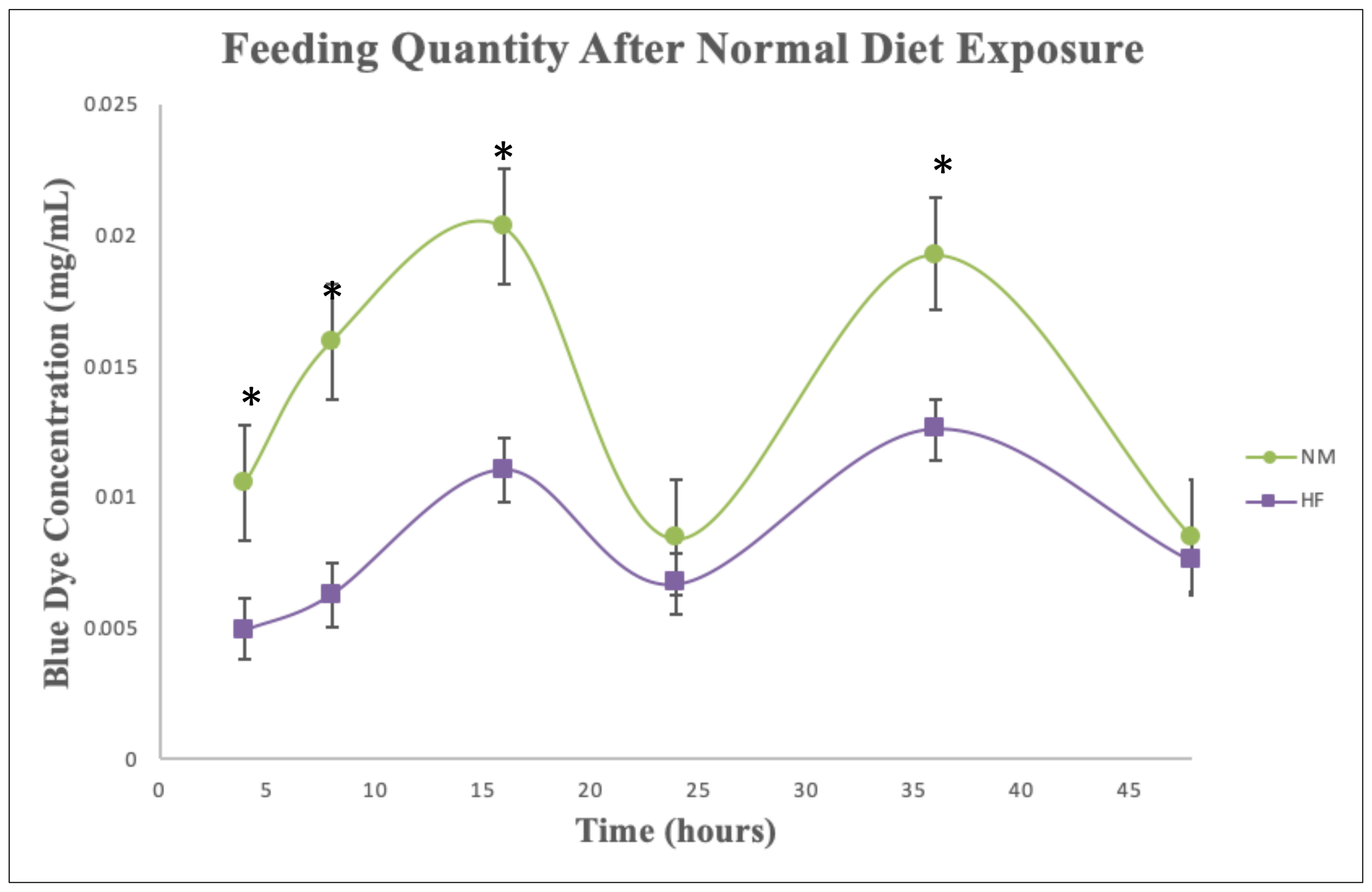

Figure 3: Effect of normal diet exposure on feeding quantity upon normal and high fat substrates. After prior exposure to a normal diet, flies were seen to present significant differences in feeding between high fat diet and normal diet at 4, 8, 16, and 36 hours as per a Student's T test. HFD feeding was seen to decrease overall compared to normal diet feeding, though increases in feeding were seen at 16 and 36 hours overall with a decrease at 24 hours. This pattern of feeding again indicates a cyclic pattern possibly related to circadian rhythm and period of activity. Error bars indicate standard error of mean (SEM).

Flies did not show a cyclic pattern of feeding when previously exposed to HFD and did not exhibit two feeding peaks as when previously exposed to normal diet.

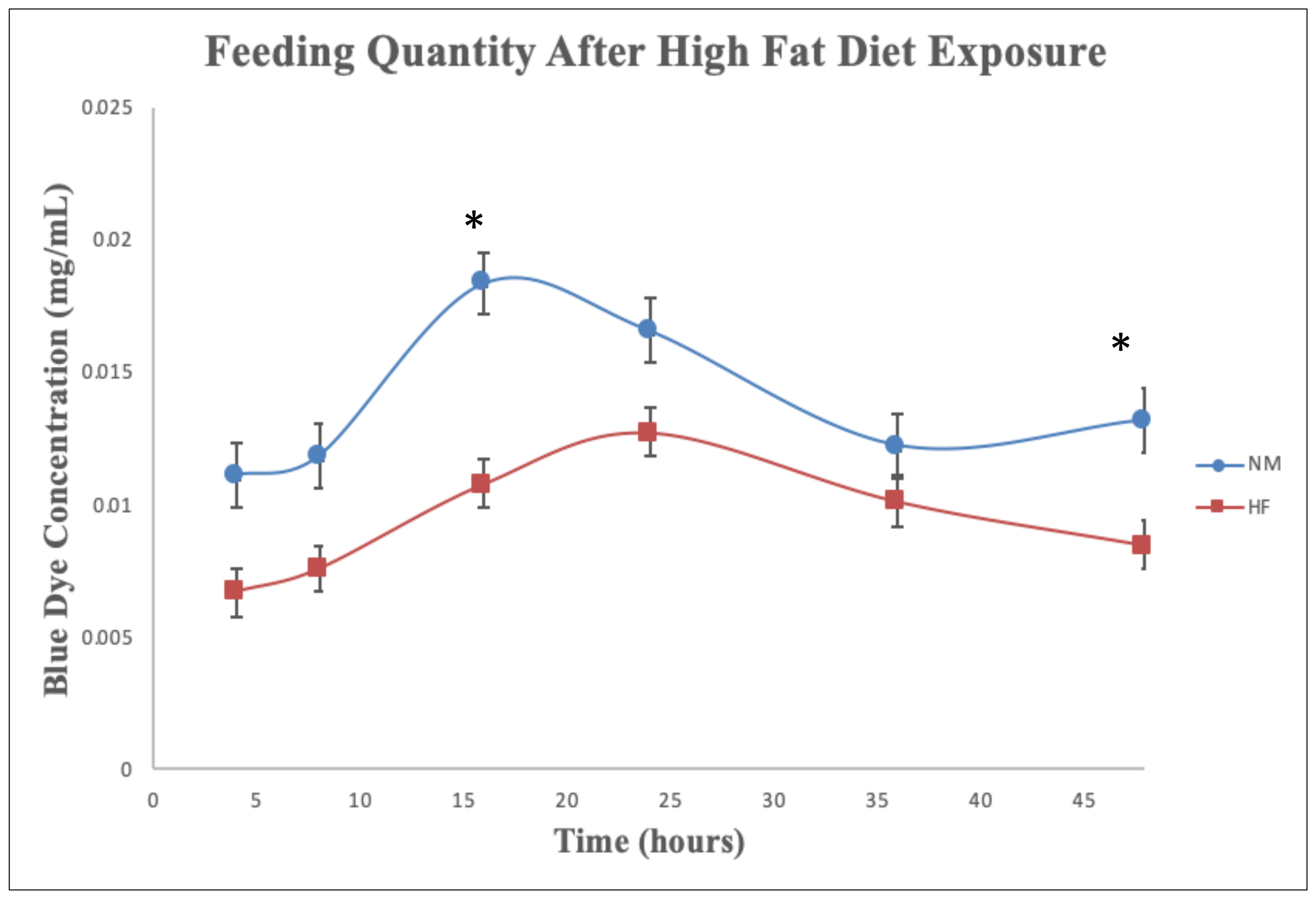

Figure 4: Effect of high fat exposure on feeding quantity upon normal and high fat substrates. After prior exposure to a high fat diet, flies were seen to increase their feeding on blue normal diet compared to blue high fat diet. Significant differences between feeding quantity were observed at 16 and 48 hours as per a Student's T test. At 48 hours, the blue NM condition was assessed using 3 replicates of 5 flies per condition apart from the typically used 5 replicates. This difference occurred due to increasing fly deaths nearing the end of the study. The cyclic pattern of HFD downregulation seen in Figure 3 is absent. Error bars indicate standard error of mean (SEM).

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Conclusion

- Flies generally increased their feeding quantity on NM compared to HSD and HFD after being previously exposed to HSD and HFD.
- Over the course of the overall 48-hour time period, flies modulated their feeding quantity on both normal diet and HSD/HFD regardless of pre-exposure.
- Flies previously exposed to NM exhibited a cyclic pattern of feeding on HSD, where significant downregulation of high sugar feeding occurred at 4 and 24 hours, but feeding was indistinguishable from normal diet otherwise.
- Flies pre-exposed to HSD did not exhibit the same cyclic pattern when exposed to HSD and NM and showed no significant differences at any time period.
- Flies previously exposed to NM also showed a cyclic feeding pattern when exposed to HFD, showing two noticeable feeding peaks over the course of the study and significant differences at 4, 8, 16, and 36 hours.
- Flies that were pre-exposed to HFD only show one feeding peak during the 48-hour period and do not show the same significant downregulation in feeding at 4, 8, or 36 hours seen in flies pre-exposed to NM. Significant downregulation did occur at 16 and 48 hours.
- Long-term exposure to obesogenic diets generally show evidence of circadian feeding disruption requiring further investigation.

Future Studies

- Adjust feeding quantity time frames and removal times to avoid possible circadian rhythm interference on feeding.
- Investigate potential circadian disruption caused by high sugar exposure using an activity monitoring system.
- Increase the high sugar diet used to 40% w/v sucrose to evaluate the effects that doubling the sugar content has on feeding quantity over a duration.
- Extend the high fat diet results to virgin females that tend to survive longer on a high fat diet.

References

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