EXERCISE AND DIET PROGRAMS DROSOPHILA SIMULANS OFFSPRING METABOLIC PHENOTYPE

by

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Abstract

Obesity in pediatric patients has increased significantly in recent decades, along with susceptibility to Type 2 Diabetes (T2D), cardiovascular disease, cancer, and mortality. The relative contribution of genetic and environmental conditions is unclear in these cases. Drosophila simulans contain an insulin-like growth pathway (IGF-1), which is a large contributing factor in anabolic stimulation and growth in children. The fat body of Drosophila is the equivalent of the vertebrate adipose tissue and liver regarding its storage and major metabolic functions. Thus, Drosophila is an ideal model to study the effects of environmental conditions, namely exercise and diet, on obesity phenotype. The purpose of this experiment was to characterize the model of obesity in Drosophila and determine how much diet and exercise act as epigenetic factors that affect metabolic function of parents and reprogram offspring phenotype trans-generationally. F0 male flies were broken into four groups, control (CF), exercise (EF), western diet (WF), and western diet with exercise (WEF), and exposed for a week. The F0 flies were then evaluated for behavioral and physical changes through monitoring locomotor activity, sleep-wake cycle, food preference, weights, and mortality. A subsection of each group was bred after exposure rather than tested, and their F1 offspring, in some instances, were tested. This process was repeated for F2 and F3. Western diet resulted in greater mortality and reduced activity in F0 and F1 flies. Locomotor data also showed increased nighttime activity in F0 western diet flies, and WFO flies from F1 and F3, suggesting a heritable disruption to the circadian rhythm. Food preference data shows that Western diet food is preferred by F0 flies that are pre-exposed to it, while control diet is preferred by those who were not. Naive F1 male and female flies with exposed parents prefer Western diet, while offspring whose parents were fed by

control diet prefer control diet. Our results indicate that obesity characteristics in Drosophila are comparable in many instances with that of humans, making them a good study model for obesity.

Introduction

Obesity in pediatric patients has increased significantly in recent decades, along with susceptibility to Type 2 Diabetes (T2D), cardiovascular disease, cancer, and hypertension (Trinh, 2013). Between 1973 and 2008 the percentage of overweight children (including obese children) aged 5 to 17 years old grew from 14.2% to 48.4% (Broyles, 2010). As of today, 18.5% of children ages 2 to 19 are obese in the United States (CDC, 2016).

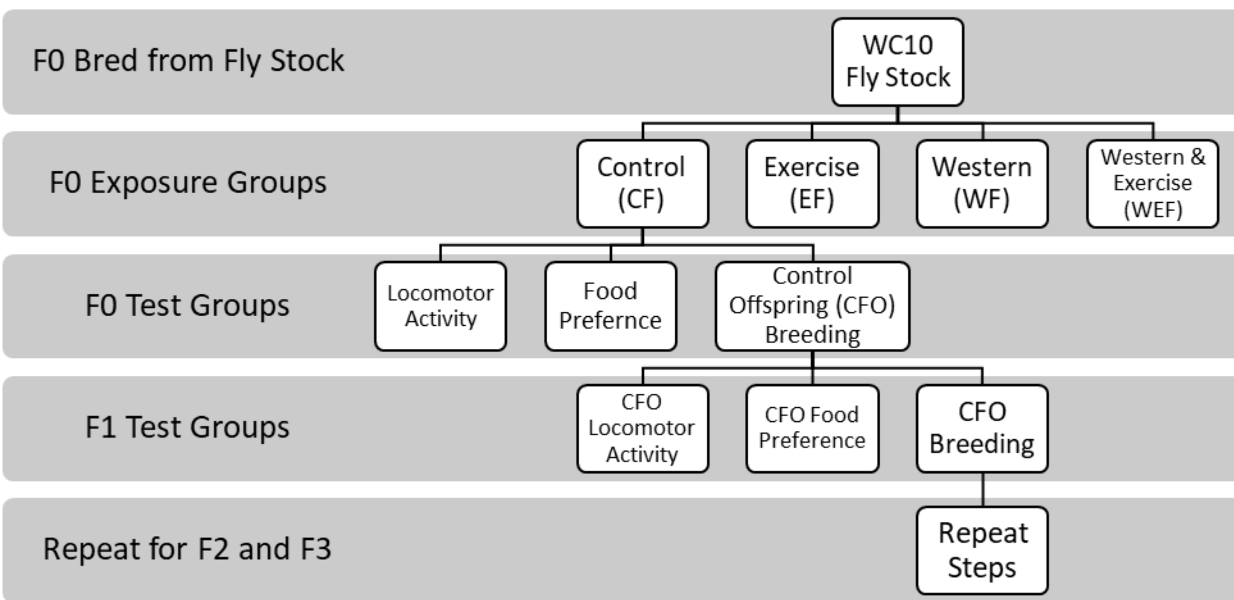
Increased rates of childhood obesity could be caused by a variety of factors, such as an unhealthy diet, low levels of exercise, anxiety, a poor socioeconomic status, and inherited genetic traits (CDC, 2016). The relative contribution of genetic and environmental factors to childhood obesity is currently unclear; however, diet, physical activity, and genetic predisposition are believed to be major contributing factors. There is an expanding body of research that suggests diet and exercise of parents can cause epigenetic modifications (stable, heritable changes) that are passed down to offspring (Hardy, T). These modifications can influence an offspring's susceptibility to obesity and cancer.

The purpose of this project is to explore the impact of a parent's exercise and diet on multiple generations of offspring using Drosophila as a test model. We identified Drosophila simulans as our ideal model because of its ability to reproduce and mature rapidly, and because its metabolic functions share several similarities with humans. These similarities include maintaining sugar homeostasis, adjusting food consumption according to nutritional need, and a similar regulatory system (Trinh 2013). Additionally, the fat body of Drosophila is the equivalent of the vertebrate adipose tissue and liver, relating to its storage and major metabolic functions.

Methods

To determine the enduring effects of diet and exercise experienced by an F0 generation on its three subsequent generations we bred Drosophila stock, formed four F0 exposure groups, tested the F0s, then bred them and tested the F1, F2, and F3 generations.

Figure 1



Drosophila breeding pairs were separated from stock in sets of 15 males and females, bottled, and bred for two days. Next, parent flies were removed and placed in new breeding bottles (repeated up to three times) to prevent excess egg layering. The offspring were allotted fourteen days to mature, then were separated by sex with a microscope (Nikon SMZ800).

Four exposure groups were established, each group composed of 3 vials of 5 males and 3 vials of 5 females. These exposure groups were control flies (CFs), exercise flies (EFs), western flies (WFs) and western exercise flies (WEFs). CFs and EFs were fed with control food that consisted of NUTRI-fly Bloomington Formulation, while WFs and WEFs were fed with "western diet" (high fat, high sugar, high salt) food made of Bloomington Formulation with added palm oil (150g per 1 liter), sucrose (150g per 1 liter), and 0.1M NaCl (5.84 g per 1 liter). CFs and WFs were housed in vials in groups of 5 in Drosophila incubators, while EFs and WEFs were moved from their vials in groups of 60 to fishbowls mounted to a timed, shaking apparatus that induced flight (exercise) every 15 minutes during the flies' designated awake hours (8AM-8PM). These four groups were exposed to these conditions for one week.

After exposure, each F0 group was broken into subgroups to be either tested via locomotor activity, food preference, climbing tests, respirometry, or breeding (Fig. 1 shows a simplified chain of events, only following CFs). Once F1 offspring (referred to as F1 CFOs, EFOs, WFOs, and WEFOs) were produced from F0 parents, the offspring were broken into test and breeding groups again. This process of breeding and testing flies without exposing them to the conditions experienced by the F0 generation was repeated for the F2 and F3 generations. Female virgins were bred with exposure or test group males to ensure the correct lineage.

description of exercise, description of your tests (food preference, loco, breeding).

Results

Our first goal and focus of fall 2019 when collecting data was to characterize the model of obesity in Drosophila through testing F0 flies. Our second goal and focus of Spring 2020 was to focus tests on F1-F3 offspring to determine how much parent environmental factors plays a role in offspring phenotype. The data presented in this document was collected from our locomotor apparatus and food preference tests.

Locomotor activity (fly movement) was recorded by loading vials of F0-F3 flies into slots in the grid of the Locomotor apparatus. The apparatus counted the number of times flies crossed an infrared beam passing through each vial and recorded the hourly movement totals for each vial over a week. Information about fly health, energy levels, metabolic rate, and circadian rhythm can be inferred from this data. For F1-F3 vials, half 3 vials per test group contained control diet food (CD), and the other half contained western diet food (WD), to see if parental exposure to western diet affects offspring response to it.

Food preference tests involved placing dyed food (red WD, blue CD) around the perimeter of a petri dish. 50-100 of same-sex flies were starved for 24 hours, then left in the petri dish with the dyed food for 2 hours. Afterwards the flies were removed, frozen, and counted according to the color of their abdomen (red, blue, purple, or blank).

F0 and F1 Results

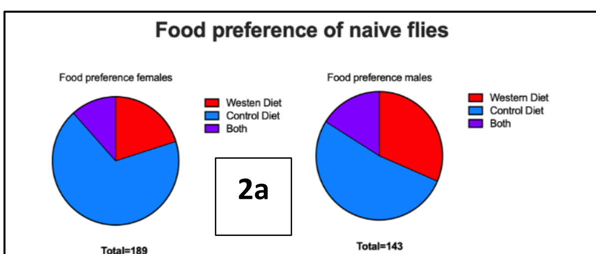


Figure 2a. F0 food preference of male/female flies after 24-hour starvation without exposure to WD. Fall 2019 Data.

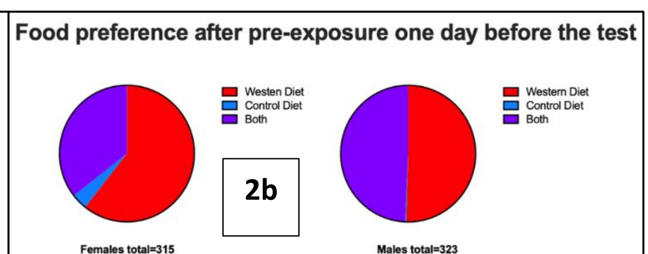


Figure 2b. F0 food preference of male/female flies after 24-hour starvation with exposure to WD. Fall 2019 Data.

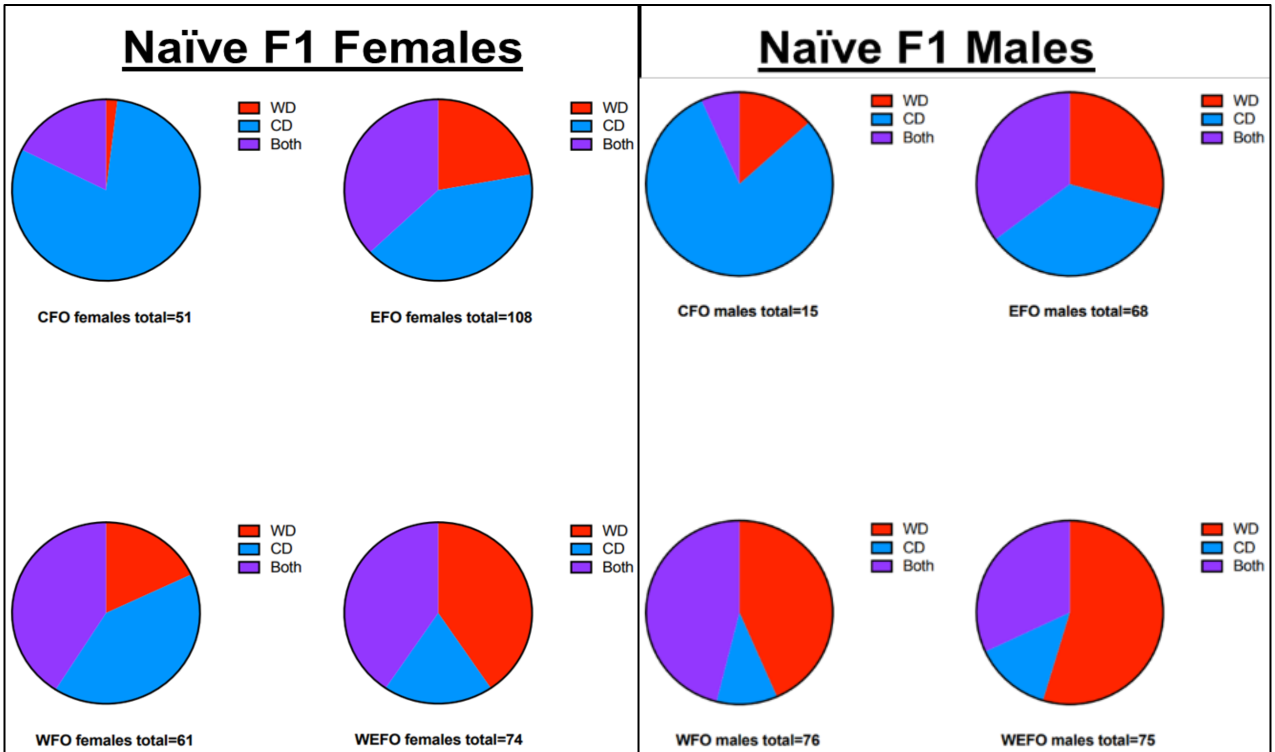


Figure 3. Naïve (no prior western diet exposure) F1 food preference, females and males from CFO, EFO, WFO, and WEFO test groups. Fall 2019 Data.

Figure 4

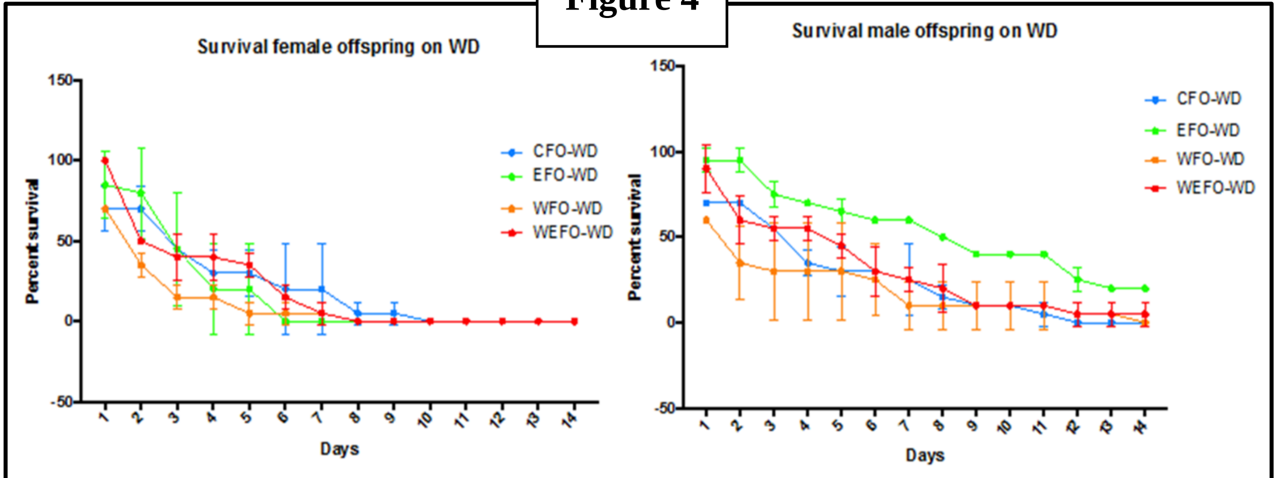


Figure 4. Survival of male and female F1 offspringflies on WD over 2 weeks.

Males survive longer compared to females living with WD.

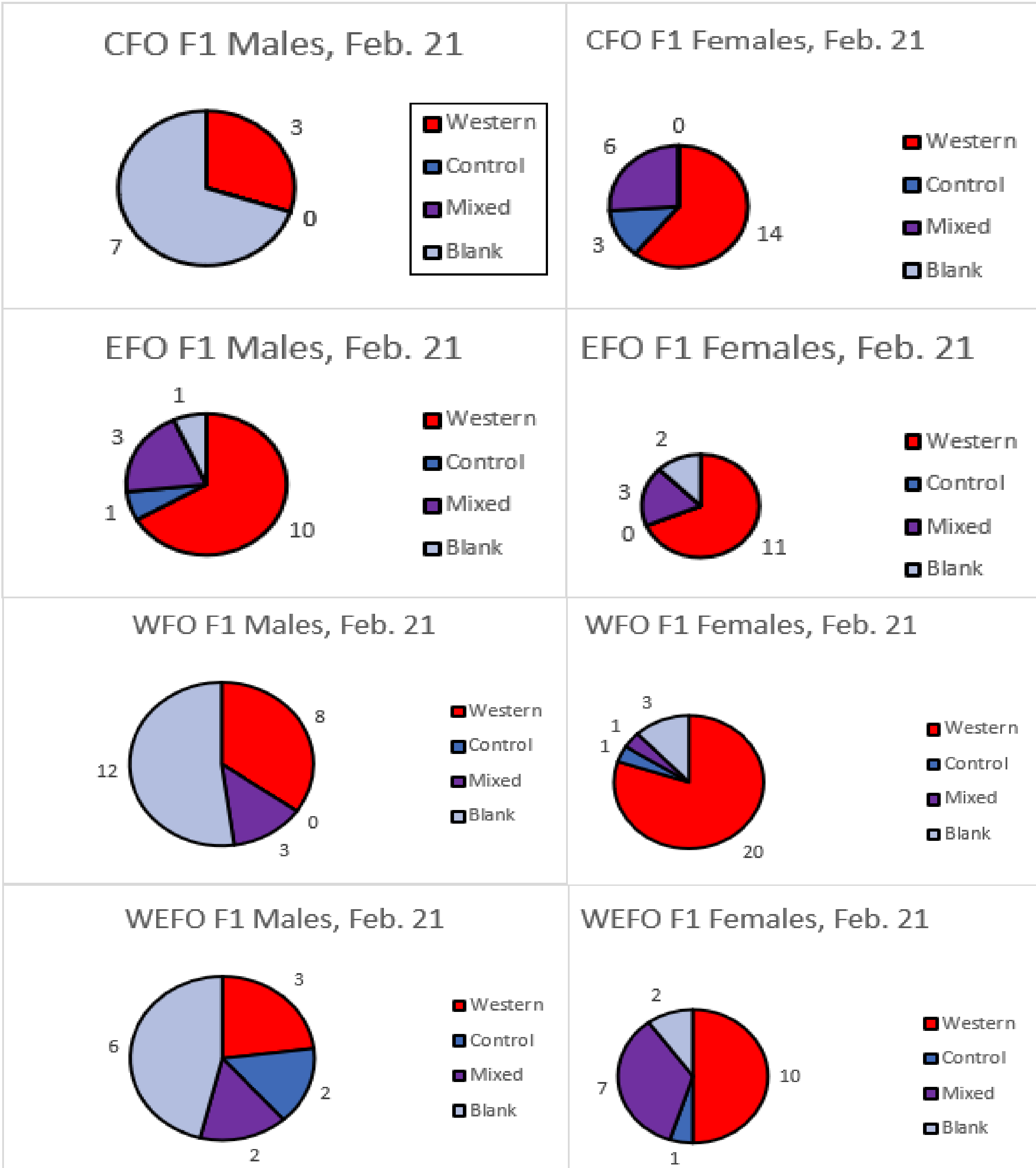
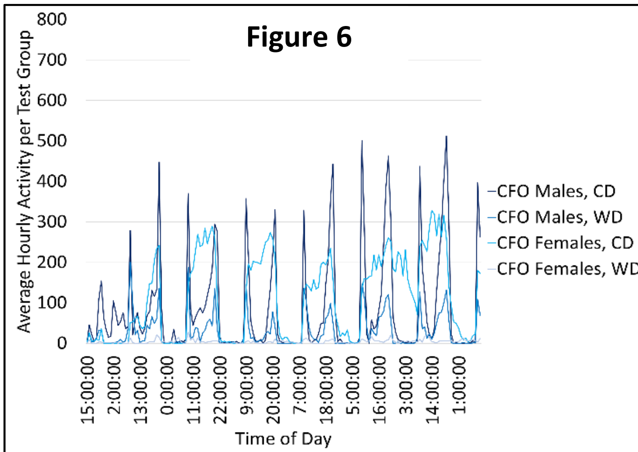
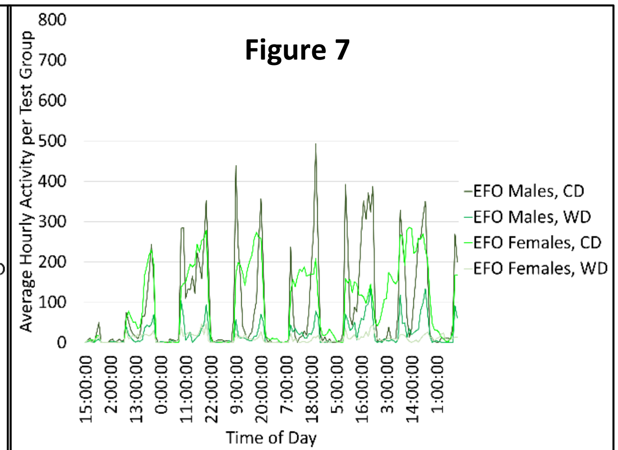


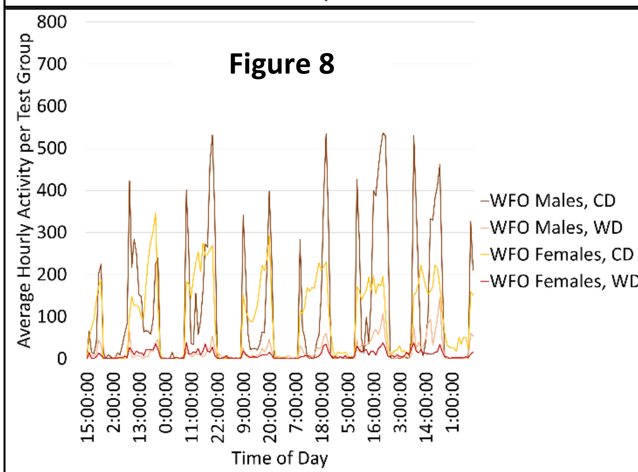
Figure 5. Naïve F1 Food Preference Data. 2/21/20.

The western portion of each chart from Figure 5 is greater than its corresponding chart from Figure 3. There is also far less control food represented in Figure 5 compared to Figure 3.

F1 Locomotor Results







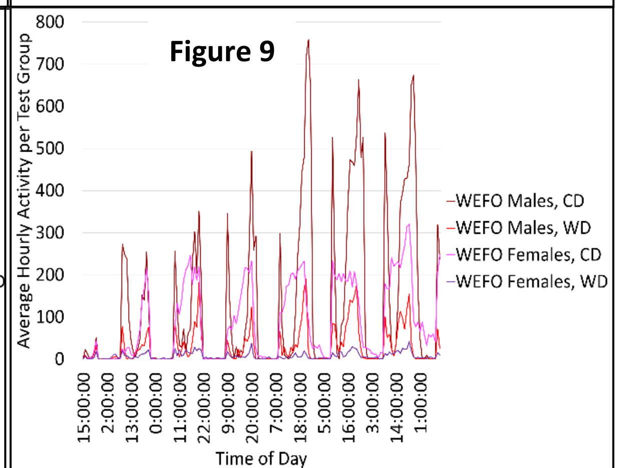


Figure 6. F1 CFO. **Figure 8.** F1 WFO.

Feb. 12-19, 2020. Average hourly activity over 1 week, Males & Females, CD & WD.

Figure 7. F1 EFO. **Figure 9**. F1 WEFO.

Feb. 12-19, 2020. Average hourly activity over 1 week, Males & Females, CD & WD.

Figures 6-9 show the average hourly activity of the four sets of F1 offspring over a one-week period. It can be observed that male flies on control diet (CD) showed the highest activity levels, while females on western diet (WD) showed the lowest activity levels. CD Females and WD males sat in the middle, with CD females showing more activity. The WEFO, CD males showed the highest activity level overall.

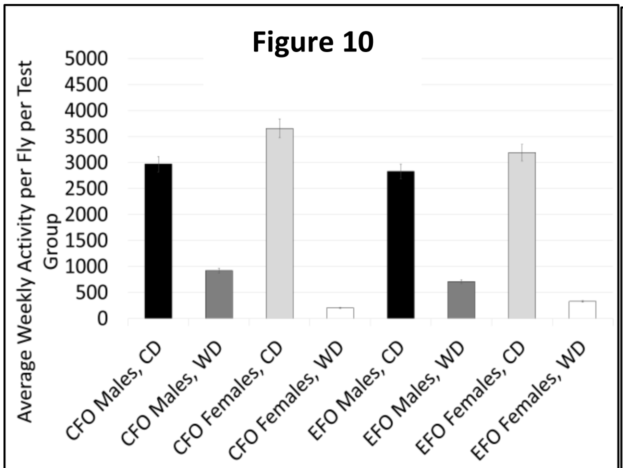


Figure 10. F1 CFO & EFO, Feb. 12-19, 2020. Average activity per fly over 1 week. Males & Females, CD & WD.

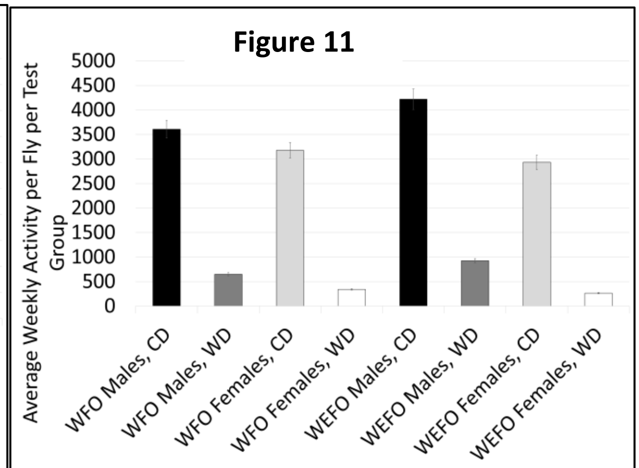


Figure 11. F1 WFO & WEFO, Feb. 12-19, 2020. Average activity per fly over 1 week, Males & Females, CD & WD

Figures 10 and 11 shows average activity levels per fly, per group over a week. Once again, F1 offspring fed on CD show greater activity compared to those on WD.

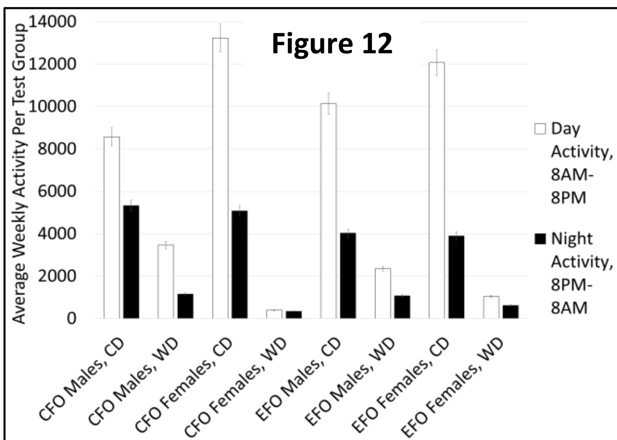


Figure 12. F1 CFO & EFO, Feb. 12-19, 2020. Average Day/Night Activity in 1 week. Males & Females, CD & WD.

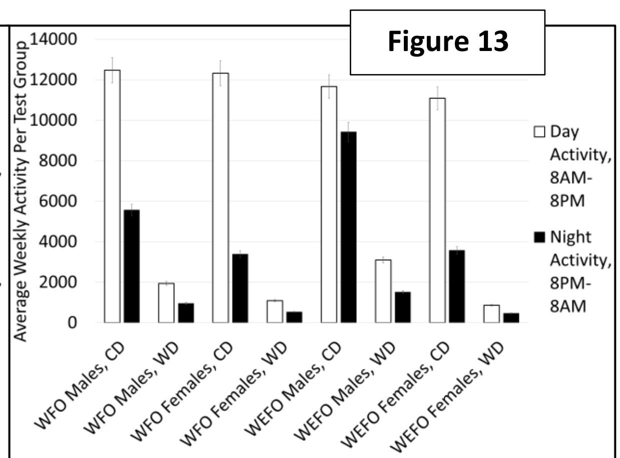


Figure 13. F1 WFO & WEFO, Feb. 12-19, 2020. Average Day/Night Activity in 1 week. Males & Females, CD & WD.

Figures 12 and 13 show F1 activity during day (awake) and night (asleep) hours. F1s fed CD show substantially higher day and night activity. F1s fed WD tend to have very close and low day and night activity levels.

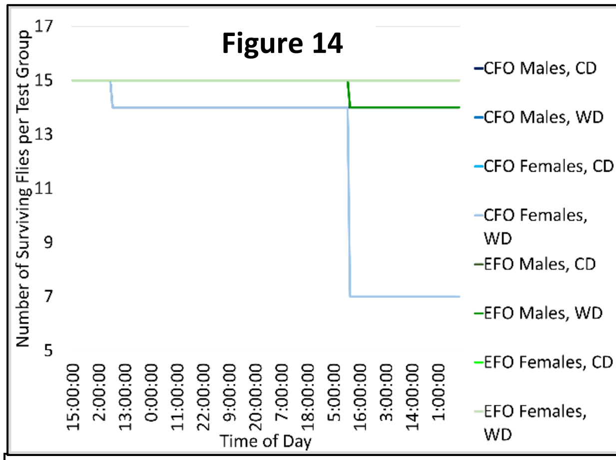


Figure 10. F1 CFO & EFO, Survivorship from Feb. 12-19, 2020. Males & Females, CD & WD.

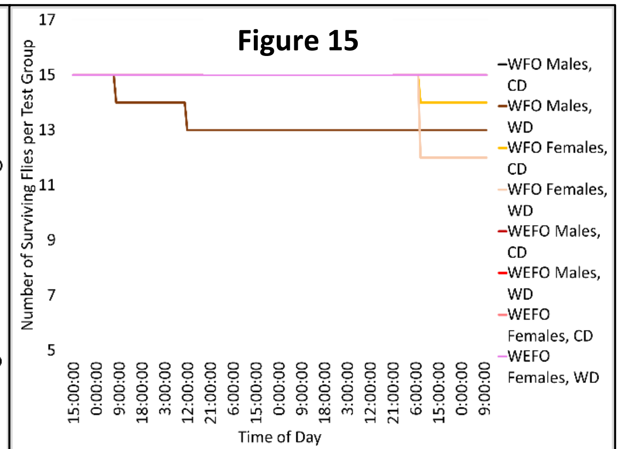


Figure 11. F1 WFO & WEFO, Survivorship from Feb. 12-19, 2020. Males & Females, CD & WD.

F1, CFO WD Females show the highest death rate in this set.

F2 Locomotor Results

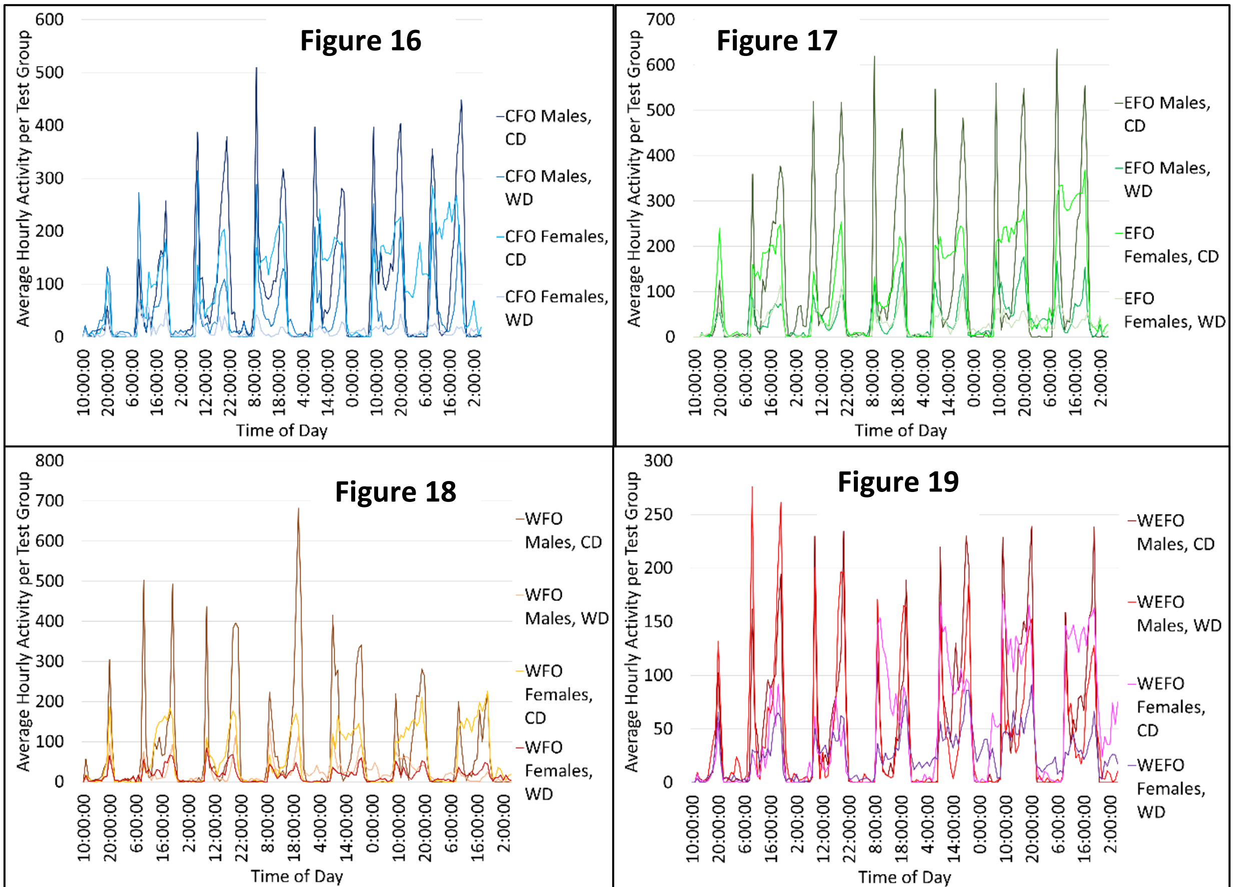


Figure 16. F2 CFO. **Figure 18.** F2 WFO. Feb. 27-Mar. 5, 2020. Average hourly activity over 1 week. Males & Females, CD & WD.	**Figure 17**. F2 CFO. **Figure 19.** F2 WEFO. Feb. 27-Mar. 5, 2020. Average hourly activity over 1 week. Males & Females, CD & WD.

Figures 16-19 indicate that male flies on control diet (CD) showed the highest activity levels, while females on western diet (WD) showed the lowest activity levels. CD Females and WD males sat in the middle, with CD females showing more activity. EFO Males on CD showed the overall highest activity levels. Most test groups show two activity peaks daily, but CD females show constant average activity levels throughout the daylight hours.

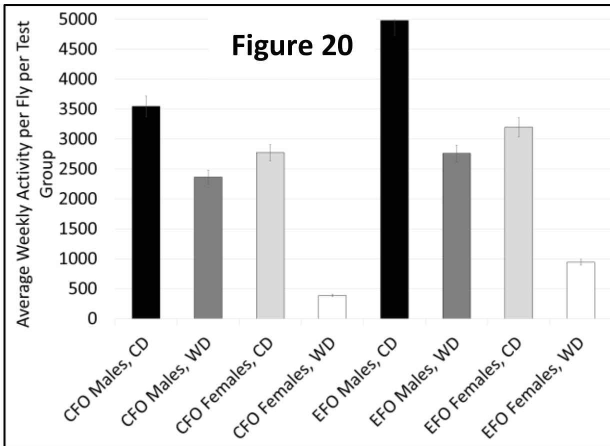


Figure 20. F2 CFO & EFO, Feb. 27-Mar. 5, 2020. Average activity per fly over 1 week. Males & Females, CD & WD.

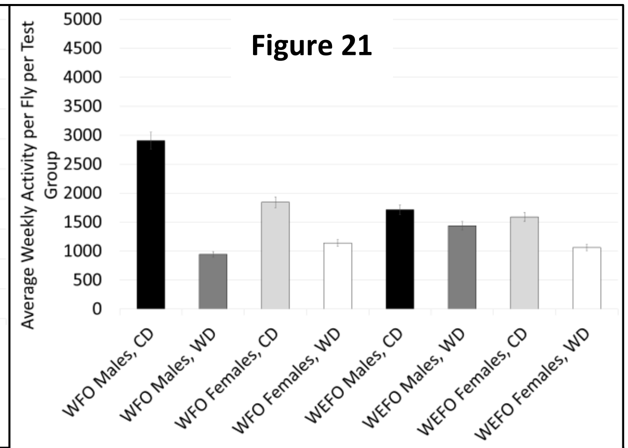


Figure 21. F2 WFO & WEFO, Feb. 27-Mar. 5, 2020. Average activity per fly over 1 week. Males & Females, CD & WD.

Figures 20 and 21 show that every F2 CD offspring is more active than their corresponding show WD offspring. CFO CD females are least active, EFO CD males are most active.

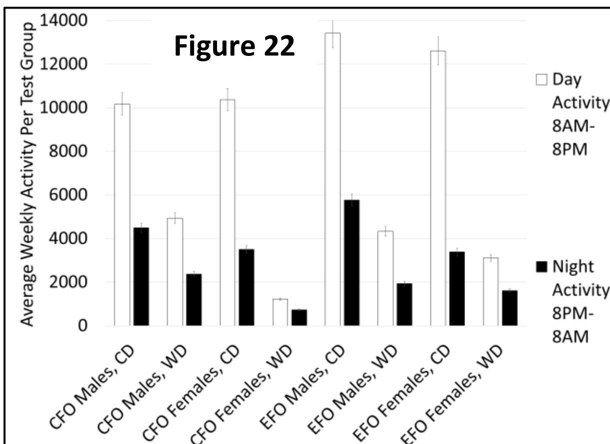


Figure 22. F2 CFO & EFO, Feb. 27-Mar. 5, 2020. Average Day/Night Activity in 1 week. Males & Females, CD & WD.

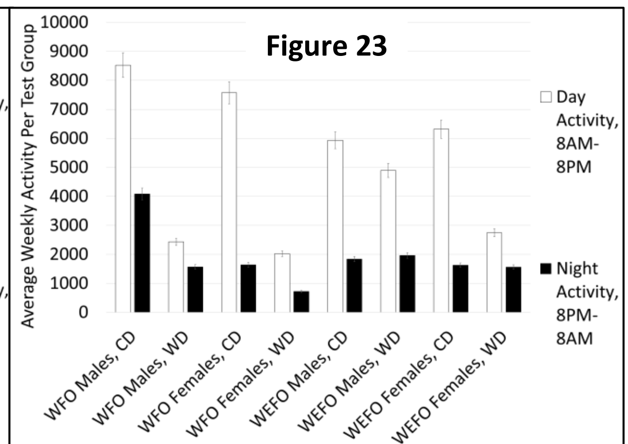


Figure 23. F2 CFO & EFO, Feb. 27-Mar. 5, 2020. Average Day/Night Activity in 1 week. Males & Females, CD & WD.

Compared to the F1 generation, F2 CFOs and EFOs show distinctly higher day time activity than the F2 WFOs and WEFOs.

Figure 24

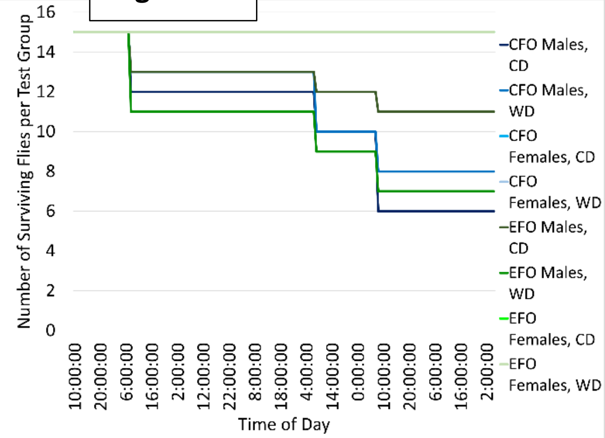


Figure 25

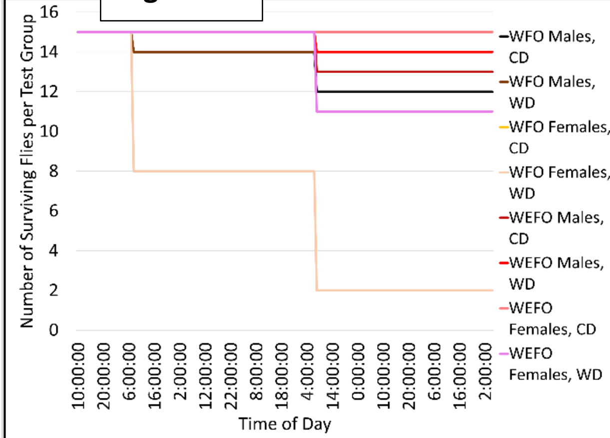
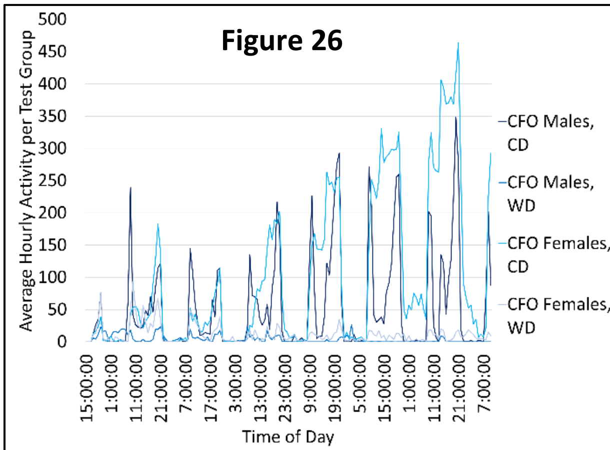
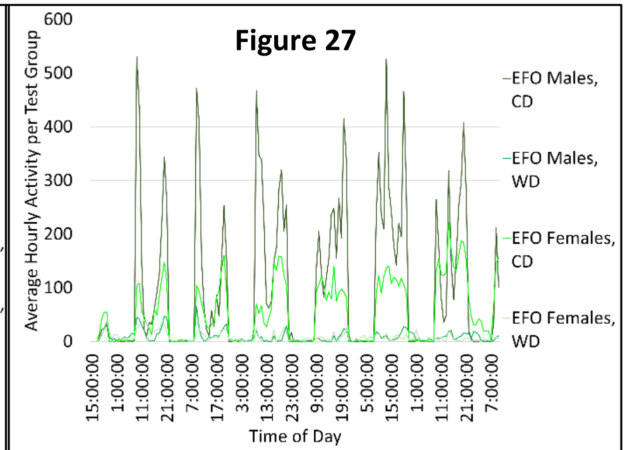


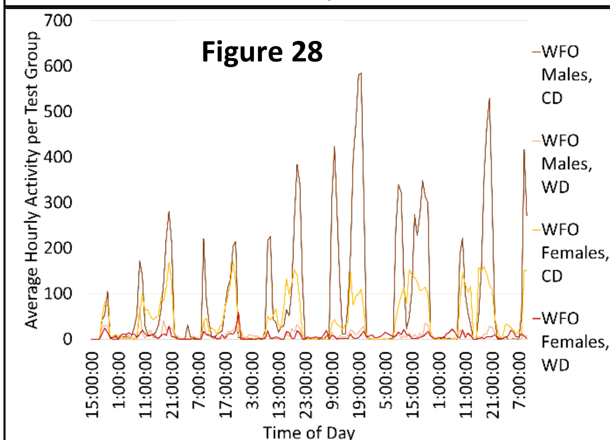
Figure 24. F2 CFO & EFO, Survivorship from Feb. 27-Mar. 5, 2020. Males & Females, CD & WD**.**	**Figure 25.** F2 WFO & WEFO, Survivorship from Feb. 27-Mar. 5, 2020. Males & Females, CD & WD**.**

F2, WFO WD Females show the highest death rate in this set and the F1 set.

F3 Locomotor Results







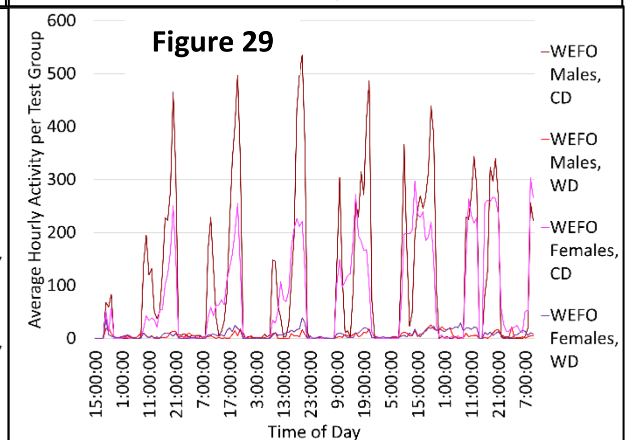


Figure 26. F3 CFO. **Figure 28.** F3 WFO. Mar 13-20, 2020. Average Hourly Activity over 1 week. Males & Females, CD & WD.	**Figure 27.** F3 EFO. **Figure 29.** F3 WEFO. Mar 13-20, 2020. Average Hourly Activity over 1 week. Males & Females, CD & WD.

Figures 26-29 indicate that once again CD males had the highest activity levels, while WD females showed the lowest activity levels. WD males in this case appear to be almost as low as WD females.

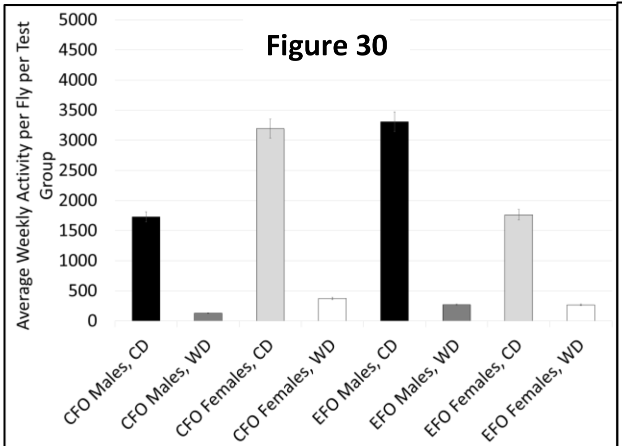


Figure 26. F3 CFO & EFO, Mar. 13-20, 2020. Average Activity Per Fly over 1 week. Males & Females, CD & WD.

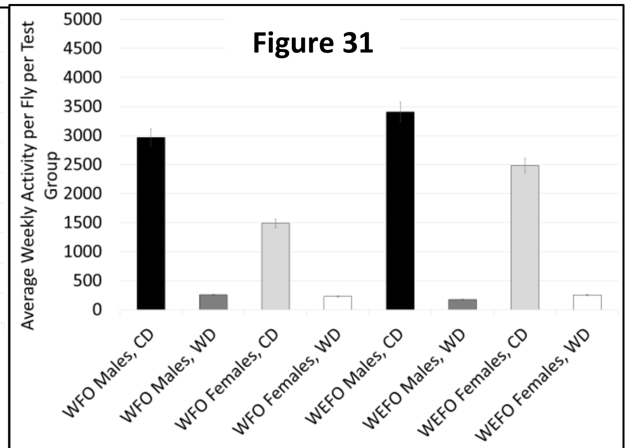


Figure 27. F3 WFO & WEFO, Mar. 13-20, 2020. Average Activity Per Fly over 1 week. Males & Females, CD & WD.

F3 CD offspring continue to be more active than F3 WD offspring, but the difference between CD and WD F3 offspring is much more substantial here than in F2 or F1.

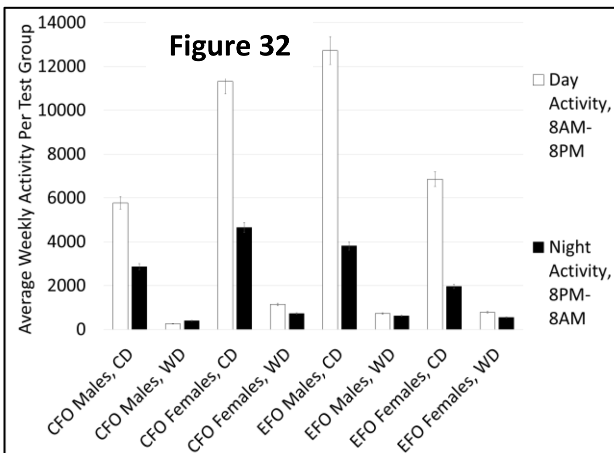


Figure 28. F3 CFO & EFO, Mar. 13-20, 2020. Average Day/Night Activity in 1 week. Males & Females, CD & WD.

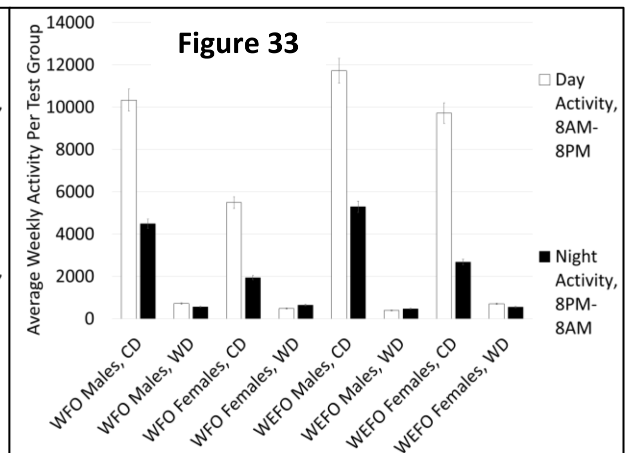


Figure 29. F3 WFO & WEFO, Mar. 13-20, 2020. Average Day/Night Activity in 1 week. Males & Females, CD & WD.

Compared to the F1 generation, F2 CFOs and EFOs show distinctly higher day time activity than the F2 WFOs and WEFOs.

Discussion

The first set of F0 and F1 data serves to establish an obesity model for Drosophila simulans (what "obesity" looks like in flies). The results show that western food tends to be addictive to flies that eat it, yet prolonged exposure appears to have a deleterious effect. Regarding weight, flies lose weight when they eat WD as fat body tissue replaces denser muscle. It is interesting to notice that EFOs survived the longest while WFOs survived the shortest on WD. Perhaps this is because the active lifestyle of EFO parents creates a bodily demand for nutrient-packed food that is passed on via epigenetic modifications.

The F0 and F1 Food Preference data together shows the addictive properties of WD after an initial exposure, and how naïve offspring will have a greater natural preference for WD if their parents were fed with western diet, or (to a lesser degree) exercised. Figure 5, containing food preference data from 2/21/20 unfortunately did not match the F1 data from last semester, perhaps due to a myriad of recipe and dye issues when preparing the food preference dishes.

The next sets of data from F1-F3 generations serve to determine how much parental environment factors plays a role in offspring phenotype. The data from figures 6-9, 16-19, and 26-29 was consistent, showing that flies on CD are more active than flies on WD, and males are more active than females (perhaps due to aggression). There is not a significant difference between F1, F2, and F3 data sets, suggesting that epigenetic modifications do not play a major role in activity levels between F1-F3 generations.

We would expect the typical sleep pattern of flies to be high activity during the day and low activity at night. We can identify the flies with irregular sleep patterns by identifying the ones that do not match our expectation. The F1 through F3 day/night data was largely consistent in showing that western offspring or flies fed with western diet tended to be more active at night compared to the other groups or have night activity levels close to their day activity levels. This slightly irregular in night activity suggests that WFO could inherit sleep irregularities from parents, or pick it up themselves from eating western diet (which is known to affect circadian rhythm).

This project faced a few limitations. Survival data was limited by the number of times in a week they were checked. Fly mortality should be done two to three times a day rather than only 3-4 entries in a week. Data collection was limited to some degree in the last weeks of this project by the COVID-19 pandemic. Early data collected from food preference tests was highly erroneous due to weak food dye and there was not enough time on campus to collect new data. Similarly, there was not enough time to collect complete F3 data, resulting in missing F3 survival data.

The next major step for this project would be to reincorporate PCR to study changes in the flies' phenotype at a molecular level. PCR would be used to evaluate change in metabolic phenotype by monitoring changes in expression of key genes like Chico, dFOXO, alpha tubulin (reference gene), and Sugar Babe. Collecting data on changes in gene expression and physical behavior due to parent's diet/exercise would paint a more complete picture of the role epigenetics plays in determining offspring phenotype.

The results of this study helped to confirm key elements of the Drosophila simulans obesity model and supports the assumption that Drosophila are a good model for studying obesity relative to humans. By continuing research into the effects of diet and exercise on Drosophila offspring, connections can be made to prevent of childhood obesity in humans. With increasing amounts of evidence supporting the role of epigenetics in childhood obesity, further research could lead to a future in which medical professionals can protect a patient's future children from obesity by correcting current environmental factors.

References

Broyles, S. et al. (2010). The Pediatric Obesity Epidemic Continues Unabated in Bogalusa, Louisiana. *AAP News & Journals*, *125*(5). https://doi.org/10.1542/peds.2009-2748

Childhood Obesity Causes and Consequences. (2016, Dec. 15). CDC. https://www.cdc.gov/obesity/data/childhood.html

Hardy, T. M. and Tollefsbol, T. O. (2011). Epigenetic diet: impact on the epigenome and cancer. *NCBI.* doi: 10.2217/epi.11.71

Henthorn, Kristina Schimmelpfeng, et al. "A role for kinesin heavy chain in controlling vesicle transport into dendrites in Drosophila." *Molecular Biology of the Cell* (2011). Document.

Trinh, I and Boulianne, GL. "Modeling Obesity and Its Associated Disorders in Drosophila." *Physiology* (2013). https://journals.physiology.org/doi/full/10.1152/physiol.00025.2012

Probst, AV, E Dunleavy and G. Almouzni. "Epigenetic inheritance during the cell cycle." *PubMed* (2009). Document.