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Effects of High-Sucrose, High-Fat, and High-Sodium Diets on Female *Drosophila melanogaster* Fertility and Health

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ABSTRACT

The obesity epidemic has become a global concern, affecting millions of people. Plummeting global fertility rates indicate a correlation between obesity and infertility, an issue that will likely worsen as the prevalence of maternal obesity rises. The effects of diet can be studied in *Drosophila melanogaster*, an ideal multicellular model with reproductive processes similar to those of humans. Obesity caused by diets rich in sucrose, sodium, or fat is known to negatively impact *Drosophila* health, decreasing egg production, shortening lifespan, and even causing transgenerational effects. Conversely, exercise may have beneficial outcomes on female *Drosophila* that are producing eggs, and its transgenerational effects are yet to be explored. By focusing future studies on female *Drosophila* affected by a combined diet, results will better replicate a Western diet consumed by women today. This literature review summarizes previous research correlating diet with detrimental effects on health and fertility to better understand the effects of a combined high-fat, sucrose, and sodium diet on female *Drosophila*. Additionally, this paper explains potential experimentation methods to implement in future studies to improve understanding of diet and fertility in *Drosophila* and its connotations to human diet and modern-day health.

Introduction

The obesity epidemic is a global concern with terrifying obesity rates that continue to increase. In America alone, obesity rates have doubled since the 1960s with almost 31.5% of adults being obese (Monaco, 2020). The popularization of high-sucrose, high-sodium, and high-fat Western diets has made obesity impossible to avoid for many. Furthermore, the complications of obesity are dangerous, causing serious cardiovascular harm, abnormal cholesterol levels, high blood pressure, and fertility issues (Segula, 2014). Infertility is also a growing concern as fertility rates have decreased almost 50% in the last century (Gallagher, 2020). Previous studies have revealed connections between obesity and infertility; however, little research regarding Western diet-induced maternal obesity has been done. *Drosophila melanogaster* is an effective model for diet-related research because the majority of processes affected by diet are evolutionarily conserved from *Drosophila* to mammals. According to Rust et al., in *Drosophila*, each ovary is composed of about 16 ovarioles, along which follicles develop to give rise to eggs. Insects like Drosophila undergo superficial cleavage through which a mass of yolk confines cleavage to the cytoplasmic rim of the egg (Gilbert, 2000). The germarium, where oogenesis begins, is a structure located at the anterior of each ovariole. Two to three germline stem cells are located at the anterior of the germarium in a niche that is produced by cap cells and a terminal filament. The stem cells divide during adulthood, producing daughter cells called cystoblasts. The inner germarial sheath cells, or escort cells, promote the early stages of cystoblast differentiation as the cells perform four rounds of mitosis to form a cyst of 16 connected cells. One germ cell then becomes the oocyte and begins meiosis while the other cells become nurse cells providing support for the oocyte. Each cyst is surrounded by epithelial follicle cells produced by follicle stem cells. The follicle stem cells produce pre follicle cells that differentiate into polar cells, stalk cells, or main body follicle cells. The budded follicles develop into a mature egg through fourteen distinct stages over four to five days (Rust et al., 2020). The process of egg formation in *Drosophila* resembles oogenesis in humans, which fundamentally utilizes similar processes.

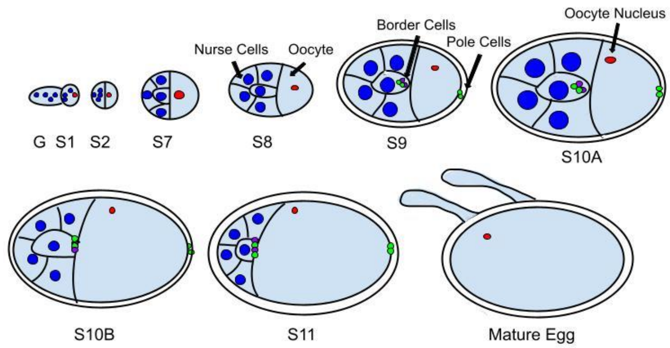

Figure 1: Model of *Drosophila melanogaster* egg maturation. Adapted from https://onlinelibrary.wiley.com/doi/10.1002/dvg.23269.

This literature review aims to explain previous research correlating diet with reduced fertility while emphasizing potential experimentation models furthering knowledge in this field. By doing so, the effects of obesity-inducing, high-sucrose, high-fat, and high-sodium diets on female *Drosophila melanogaster* fertility and overall health can be understood at a molecular level.

Literature Review

The detrimental effects of excess sucrose are known, including cardiovascular problems, obesity, and an increased risk of diabetes. Jenson et al. explain that normally, the liver and fat cells metabolize sugar and convert dietary carbohydrates into fat. A surplus of sucrose created by diet is not metabolized, causing fat accumulation, increasing the risk of heart and fatty liver disease (Jenson et al., 2018). The effects of sucrose on fertility are explored in a 2017 study by Brookheart et al. focused on sucrose-induced maternal obesity in *Drosophila melanogaster.* The sucrose-rich diet promoted dangerous insulin resistance while disrupting ovarian metabolism and function. A stock of virgin female flies was divided and fed either a high-sucrose or normal diet. The females were mated with male flies after 7 days of feeding. Female flies on a high-sucrose diet (HSD) had ovaries 28% smaller than the control group. The high sucrose feeding reduced the number of eggs laid by 62% in the HSD group compared to the control. In the HSD offspring, a decrease in offspring number existed at the larval, pupal, and adult stages. Furthermore, decreased ovarian size and impaired egg maturation were observed, indicating that a HSD significantly hinders female fertility. The negative effects of the HSD hinder offspring development and survival for several generations after the initial obese subject, demonstrating the transgenerational impacts of sucrose-induced obesity (Brookheart et al., 2017).

Another study by Chandegra et al. analyzing the effects of dietary sugar on lifespan and starvation resistance reveals that a HSD in *Drosophila melanogaster* causes sexually dimorphic consequences on several factors of health. *Drosophila melanogaster* females and males were fed HSD diets to observe their feeding habits change to accommodate reproductive/health needs per individual. The study explains differences between *Drosophila* sexes in their responses to sugar-rich diets and how the variance ensures improved reproductive and survival rates for the individuals. Females increase intake on diets with high protein and low sugar content while males feed on sugary diets. These differences are caused by sex-specific reproductive needs to ensure effective reproduction; females require high levels of protein to develop eggs and healthy offspring. The females' reduced feeding caused by low-protein diets would create a larger decrease in protein intake, negatively affecting egg production and offspring development. A starvation resistance response may have evolved in females to allow them to survive food shortages during reproductive periods (Chandegra et al., 2017). Furthermore, a 2013 study by Lushchack et al. found that the carbohydrates fructose, glucose, and sucrose decreased lifespan by 13 to 27%. Sucrose significantly decreased fly fecundity and presented a higher mitochondrial protein density compared to the control group. This study also displayed impaired egg development, which is in agreement with previous studies (Lushchak et al., 2013).

To further understand the transgenerational effects of a high-fat diet (HFD), Dew-Budd et al. used *Drosophila* melanogaster to investigate the genetic impacts of a HFD on three generations. *Drosophila* were divided into different groups and fed either a normal diet or a HFD. The flies were mated and eggs were collected, raised, and bred again. The study noted several interactions between genotype, and sex in the second and third generations. Both male and female pupal weight had genotype effects, or average effects associated with an allele, for future generations, solidifying the transgenerational effects of diet-induced obesity. Furthermore, the initial and third generations displayed a significant genotypic effect on egg size. Transgenerational responses to initial diets varied between genotypes and sexes. Overall, moderate correlations between the parents' initial reaction to a HFD and the offsprings' phenotype were noted (Dew-Budd et al., 2016). The association suggests the possibility of detrimental effects occurring in descendants regardless of the presence of phenotypes in ancestors exposed to damaging diets. Therefore, the HFD may display negative phenotypes in some generations while leaving no observable impact on earlier generations.

The diminished offspring development caused by the transgenerational consequences of high-fat-induced obesity may affect fertility rates in future generations, creating additional issues. The popularized Western diet (WD) is notorious for its fatally high sucrose, fat, and sodium content. However, exercise has been effective at preventing health deterioration when paired with obesity-inducing diets. To understand these reversing effects, a study by Murashov et al. tested the effects of a high-fat, high-sucrose, high-sodium, and Western diet on fly health. The study used groups of male and female flies and fed groups different diets and tested their health. A group of male flies exercised for 7 hours a day on a motor platform and the flies' strength was measured through a climbing activity. The study found that the WD decreased life span significantly, damaged fertility, and harmed embryos. Furthermore, a WD high in saturated fat, sugar, and salt is more harmful to lifespan, locomotor activity, and fertility than the separate components alone. This information indicates that diets popular today are more damaging than previously considered. However, regular exercise was able to reverse most of the detrimental effects caused, even allowing WD flies to become stronger than the control group. Exercise also significantly reduced the levels of triglycerides (TAG) in WD flies compared to the control group (Murashov et al., 2020). Knowing the negative effects of a WD's components on fly health and fertility, it can be assumed its effects are equal or increased in female Drosophila producing eggs.

Besides affecting fertility and increasing the chances of heart disease and diabetes, a WD can negatively affect sleep. Sleep fragmentation can increase vulnerability to respiratory infections and chances of cardiovascular disease development while weakening immune systems. A study by Xie et al. analyzed the effects of a high-sodium diet (SD) on sleep mechanisms of *Drosophila melanogaster*. The *Drosophila* used for the study were divided into groups and fed a SD or a normal diet. Sleep patterns were measured in terms of sleep duration, activity breaks between sleep, and mean episode duration. The researchers found that a SD caused young *Drosophila* to display fragmented sleep similar to that of significantly older individuals. To test the effects of diet salt concentration on food preference, flies were fed 1% sodium chloride or normal food. Food consumption was similar for both groups, demonstrating that sleep phenotypes such as fragmented sleep were caused by a SD and not decreased food consumption. Sodium-rich diets create alterations in the dopaminergic pathways that release dopamine, leaving damaging effects on sleep patterns. The damage caused resembles the deterioration caused by natural aging, hinting at potential aging outcomes of the diet affecting other components of health. (Xie et al., 2019).

Contrarily, a 2016 study by Reis explores diets that positively affect *Drosophila* fertility and health. The study avoided high sodium or fat diets to ensure positive diet-induced effects, reiterating the negative effects of a WD. Adult fly stocks were allowed to lay eggs on grape juice plates with yeast paste for 4 to 6 hours. One day after hatching, 50 larvae were transferred to vials of synthetic mediums. The synthetic diet used in the study increased sucrose concentration 1.75-fold and 4 times the normal carbohydrate, protein, and lipid concentrations were included. The study revealed that this diet increased larval development and eclosion rates without causing a significant change in TAG levels. Fat body, a tissue functioning in energy storage, metabolism, and immune response in Drosophila, was analyzed to study fat accumulation. (FB) dissection in the larvae revealed excess lipids inside FB cells, suggesting that diets rich in carbohydrates and lipids promote fat accumulation. The study also found that *Drosophila* fed a protein-rich diet displayed a reduction in feeding, indicating that the protein content of diets triggers satiety signals. Maintaining a normal yeast: sucrose ratio was vital for optimal fly lifespan, indicating that both an increase and decrease in sucrose can be detrimental to fly health. However, a high protein diet was able to positively affect lifespan, even when paired with high sucrose levels (Reis, 2020). To summarize, protein-rich diets ensure improved reproduction and egg development, which have a positive effect on health, allowing the fly to withstand high sucrose levels.

In multicellular organisms, insulin/IGF signaling (IIS) works to match energy needs with storage in metabolic homeostasis, growth, and reproduction. IGF, or insulin-like growth factor, is a hormone promoting tissue development. *Drosophila* insulin consists of insulin-like peptides called Dilps that function in development and metabolic homeostasis. To understand the effects of a high-sucrose diet on insulin resistance, a 2012 study by Léopold and Pasco studied Dilp resistance. The study fed two groups of flies either a normal diet or a diet with 5 times the recommended amount of sucrose. The HSD flies were given more time to develop. This study found that a HSD caused growth inhibition in *Drosophila* as a result of peripheral Dilp resistance. Dilp-resistance causes metabolic disorders with phenotypes similar to those seen in patients with Type II diabetes. Neural Lazarillo, a secreted protein homologous to the protein correlated with Type II Diabetes, is also increased with diet-induced Dilp resistance. The diet also affected egg development and pupal growth; the HSD flies produced smaller flies, indicating that Dilp resistance affects growth functions as well (Léopold & Pasco, 2012). The study indicates that maternal obesity caused by a HSD causes developmental delays in offspring.

Previous studies have confirmed correlations between diet-induced obesity and damaged *Drosophila* fertility and overall health. However, research regarding the specific effects of Western diet components on female *Drosophila* reproductive health has been vague and limited. To further study the connections, the consequences of a combined diet on females in varying stages of reproductive age must be explored. Studies have proven that a combined diet is more dangerous than each of the ingredients alone, and the diet most closely represents popularized Western diets, revealing additional information regarding the diets consumed today. Comparing the diet's impact on virgin females, and *Drosophila* post egg production flies may reveal what aspects of female reproduction the diet affects most. The transgenerational consequences of diet-induced obesity have revealed that the detrimental effects of obesity are not short-term, and are seen in several future generations. Lastly, starvation resistance in females should be explored further to understand the mechanisms behind starvation mechanisms activated by high-sugar and low-protein diets. Comprehending these molecular mechanisms may reveal more information regarding reproduction mechanisms that ensure offspring health and strong egg production.

Methods

In a perfect setting, with practical resources and time, obesity-inducing diet administration in *Drosophila melanogaster* would be implemented to understand the effects of obesity on offspring health and pregnancy complications. The experimentation would be modeled off of Dr. Murashov and their team's study on the effects of high fat, sugar, and salt diet in wild-caught *Drosophila simulans*. The study derived fly stock from 6 *Drosophila simulans* isofemale lines from Greenville, NC. The fly stock was sustained on the standard Bloomington Formulation diet in a controlled environment at 24 ℃ with 70% humidity under a 12-hour light-dark cycle to replicate their natural environment. Experimentation was performed on age-matched 3 to 4-day-old flies. The flies were raised on the standard Nutri-Fly Bloomington diet until their diet was adjusted at age 3 to 4-day old. The flies were transferred to the Western Diet, a combination of high sugar, fat, and sodium content, a high-fat diet, a high-sucrose diet, a high-sodium diet, or kept on the standard Nutri-Fly Bloomington diet (CD). The WD included 15% Nutiva USDA Certified

Organic, non-GMO, Red Palm Oil, 15% sucrose, 0.1 M NaCl; HFP-15% Nutiva USDA Certified Organic, non-GMO, Red Palm Oil. The HSD contained 15% sucrose and the SD consisted of 0.1 M NaCl. The diet used in the study caused several deleterious effects as explained in the literary review, making it a viable choice for a future experiment. Therefore, in a potential setting, the experiment can be repeated with similar dietary changes with a female-focused test with female *Drosophila* of varying ages.

For data analysis and imaging purposes, a strategy similar to Dr. Brookheart and their team's study on the effects of sucrose-induced obesity on ovarian function would be utilized. To photograph ovaries, the study isolated ovaries from both groups of flies and placed the specimen on glass slides with PBS. The ovaries were viewed with a Zeiss fluorescent microscope equipped with an Axiocamera. The number of stage 14 eggs was counted while mitochondrial size and total ovary area, length, and width were determined using ImageJ. For electron microscopy, ovaries were fixed in Modified Karnovasky's Fixative solution immediately after isolation. The ovaries were processed for electron microscopy by the Washington University Research Electron Microscopy Core. A JEOL 1200EX electron microscope was used to view sample images acquired via a high-resolution CCD-based camera. After 7 days of feeding, female flies were mated with males for 18 hours. Eggs produced were counted and supplied with proper nutrition. TAG and glucose were measured in isolated ovaries by rinsing the specimen in PBS and homogenizing the ovaries in PBS + 0.1% Tween-20 after dissection. To calculate glucose measurements, 2 µl of homogenate was added to 98 µl of Infinity Glucose Hexokinase Liquid table Reagent (Fisher Scientific), incubated for 15 minutes at 37 ℃. After inactivating lipases through incubation, and 2 µl was added to 198 µl of Thermo Infinity Triglyceride Reagent (ThermoFisher Scientific), ovarian TAG measurements were taken. Ovary cholesterol concentration was measured similarly using the Amplex Red Cholesterol Assay Kit and microplate reader.

These data analysis methods were effective at displaying the effects of sucrose-induced obesity, so the methods should be implemented in future experiments. To further understand the specific effects of a combined diet on female *Drosophila* fertility, total ovary, TAG, and glucose measurements should be calculated at several stages of life. Calculating ovary length at three or more stages of female *Drosophila* life would elucidate the long-term effects of diet on reproductive health while revealing the stages of life that are most affected by diet-induced obesity. Furthermore, implementing a larger sample size of *Drosophila*, including wild-caught simulans and lab-bred specimens would create a more diverse experimentation group. Implementing these strategies can provide a more informational outlook on female *Drosophila* fertility and diet-induced obesity while revealing additional effects of the popularized Western diet.

Summary of Conversation with a Scientist

The author talked with Dr. Daniela Drummond-Barbosa, a professor studying dietary effects on fertility in *Drosophila melanogaster* at the Johns Hopkins Bloomberg School of Public Health.

The use of *Drosophila melanogaster* is vital when researching the effects of diet on fertility, for the model organism has cellular and reproductive functions extremely similar to those in humans. Fundamentally, the multicellular organisms are the same, so effects seen in *Drosophila melanogaster* can be expected in humans. Flies are about 60% homologous to humans and reproduce at fast rates, allowing researchers to comprehend the transgenerational effects of diet. Furthermore, fat distribution in *Drosophila* aligns with body fat placement in humans. Despite the relevance of *Drosophila* experimentation to humans, diet must be studied in other mammals to improve understanding of diet-induced obesity. Further research can be done in mammals like mice or monkeys, creating a more accurate display of diet-induced obesity.

Based on the stage of development, diet can have varying effects on *Drosophila*. Dr. Drummond-Barbosa states that metabolic needs are very different in different stages. For instance, embryos do not encounter much environmental variation besides air quality and temperature, so diet does not have a large impact on embryos. Larval stages are completely different, for larvae eat large volumes of food and increase body mass by approximately 1000-fold over 3 stages. Adults are no longer increasing their body mass, yet adult *Drosophila* must produce reproductive cells, which is a specially demanding process in females. Adult males do not require as much food as female *Drosophila* because sperm production does not require as many resources and energy as in the case of females. In females, egg production requires a large production of biomass in the form of eggs (filled with building blocks for the development of the future embryo), such that females will increase their food consumption.

Egg production involves high-protein amounts, which is why female *Drosophila* consume protein-rich foods during egg production. When considering the effects of diet on varying stages of life, it is safe to assume that diet will distinctly affect males and females of differing ages.

Discussion

Research in female *Drosophila* studying the effects of diet has been limited, but related studies have displayed a strong correlation between diet-induced obesity and reduced ovarian function. This paper utilizes previous papers and studies to comprehend the effects of a combined high-sucrose, sodium, and fat diet on female *Drosophila* fertility. Considering the differences between males and females regarding consumption habits and reproductive needs, diet will have varying effects on females in different stages of development. Female *Drosophila* that are producing eggs would be affected significantly more than females in the embryo stage due to variations in dietary requirements. By studying the effects of a combined diet, experimentation in *Drosophila* can better replicate popular human diets, providing thorough insight into the effects of sucrose, sodium, and fat. Understanding maternal obesity is vital to fully comprehend the transgenerational effects diet has on fertility and health. The transgenerational effects of a high-sucrose diet are clear, impacting larvae generations after the initial obese subject, yet the effects of a combined diet are yet to be explored. A combined diet has been more deleterious to *Drosophila* health than the ingredients alone, indicating that increasing all three factors may have a more devastating transgenerational effect. Male *Drosophila* health can be positively affected by exercise despite a combined diet, elucidating that exercise may mitigate the impacts of diet-induced maternal obesity. Implementing a flight-exercise experiment in female *Drosophila* in varying stages of development may diversify the understanding of positive exercise consequences. Exercise may be able to reverse the detrimental effects of maternal obesity while improving the health of offspring in a transgenerational manner. The positive effects of exercise may be seen in future generations, resembling the negative long-term consequences of diet-induced obesity. More research utilizing a varying age range of *Drosophila* while testing a combined diet must be implemented in the future. Studying maternal obesity in *Drosophila* will reveal information regarding oogenesis defects caused by diet, which can provide insight into infertility in humans. Exploring this realm of scientific testing will create an improved understanding of the correlations between the obesity epidemic and plummeting fertility rates while elucidating the long-term effects of sedentary behavior and Western diets.

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