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The Effect of Overnutrition on Mediated Collagen in *Drosophila Melanogaster* Physiology

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ABSTRACT

Obesity affects approximately 13% of the adult population, resulting in excessive fat cell deposition and metabolic dysfunction. Collagen, a protein that supports skin regeneration, muscle building, and pain relief, is studied in Drosophila melanogaster under different diet conditions. This experiment aims to test whether overnutrition affects physiology and collagen due to any anatomical changes in Drosophila Melanogaster, more commonly known as fruit flies. The research conducted over several weeks utilizes two diets: an obesity-inducing diet containing excessive glucose and coconut oil and a traditional diet of blue food medium, yeast, and water. The study groups include a wild-type, collagen-mutated, and obesity-mutated group, with multiple assays measuring how the obesity-inducing diet affects each population and their collagen levels. The results reveal that the obese group experienced higher food consumption levels but had lower locomotive ability than the other test groups. Hydroxyproline, the building amino acid for collagen, and collagen levels were higher in the obesity mutant than in the wild-type and collagen mutated group. The study shows that anatomical changes in organisms are influenced by both diet and movement. The obesity group, on an obesity-inducing diet, experienced decreased movement related to increased food intake and decreased neural activity. The high-fat and high-sugar diet suppressed neuronal autophagy, created inaccurate hunger and satiety perceptions, and increased collagen deposition. Anatomical changes were observed in collagen-heavy tissue areas, whereas decreased neural activity and increased feeding rates were behavioral changes. The study emphasized the importance of a healthy diet and exercise in promoting overall health.

Introduction

Nutrition is essential for the survivability of all animals. A balanced diet is vital because it gives organisms energy to stay active throughout the day and stimulate bodily functions. Typically, a healthy diet consists of vegetables, fruits, grains, and protein. When healthy eating habits are followed, the organism's body can grow, repair, and maintain itself. However, overnutrition is a form of imbalance in nutrition with excessive food intake, leading to fat accumulation and nutrient deficiency. Severe overnutrition will then lead to obesity, where excess amounts of lipids cells are produced. In particular, *Drosophila Melanogaster,* more commonly recognized as the fruit fly, gain nutrients through fermentation, sugary substances, and rotting vegetation. They adapt to new environments; adaptations can help exploit foods of differing physical properties or tolerate new, frequently toxic chemical compositions. Like in humans, overnutrition in flies can affect lipid cells, leading to a metabolism disturbance in the body. As *Drosophila melanogaster* has a set range of lipid cells, an increase in these cells would also be comparable to obesity in humans (Baenas & Wagner, 2022).

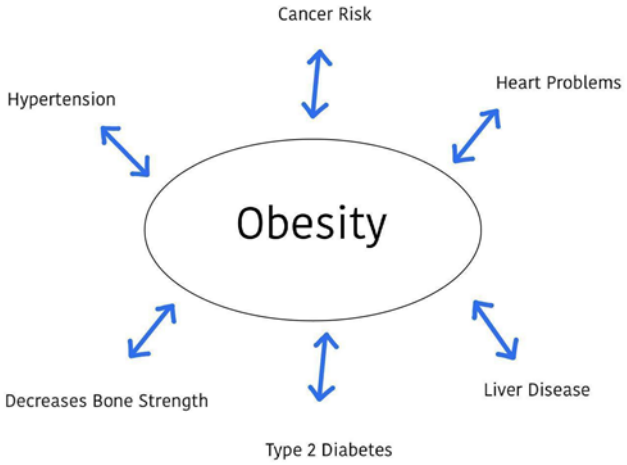

Figure 1. Obesity and Its Relationship With Other Negative Health Impacts

Overnutrition can lead to obesity and severe health concerns such as diabetes, hypertension, and degenerative bone diseases (Kyrou et al., 2018). In mouse models, researchers have found that obesity decreases bone stiffness, weakening cartilage and skeletal structures. In addition, overnutrition was regularly associated with factors among specific school types, sex, snacking habits, and sweet food preferences (Belay et al., 2022). Directly in countries like Ethiopia, overnutrition and its correlation to obesity have become a massive health concern, and programs and organizations must be sought out to help fight the negative impacts.

Collagen is an extracellular matrix protein that provides muscle buildup and flexibility. Specifically, collagen is abundant in the adipose tissue, a fatty connective tissue. The extracellular matrix of adipose tissue contains multiple types of collagen, such as collagen I, IV, V, VI, VII, VIII, and IX (Pasarica et al., 2009). When an organism is exposed to higher quantities of nutrients, eventually leading to obesity, the body generates a higher quantity of adipose tissue (Gómez-Hernández et al., 2016).

When high amounts of fats and sugars are consumed, and physical activity is not used to burn off the excess energy, the body stores the abundance of energy contents within the body (Ludwig & Ebbeling, 2018). These excess contents of energy are stored in the form of lipids. Lipids are fatty, oily compounds that are essential building blocks in cells. However, excess lipids can increase the risk of heart attack and stroke because blood cannot flow as easily. Collagen IV, an example of an assembly and incorporation of structural protein, is critical to heart integrity and overall functionality (Wilmes et al., 2018). This demonstrates how collagen IV correlates to excess lipids cells, a symptom of obesity.

Furthermore, different levels of consumption regarding nutrition may initiate variations in collagen production, leading to adverse effects. Muscles and tissues may become dysfunctional due to decreased collagen production, and bodily functions will be less efficiently performed. This dramatically impacts the structural balance of an organism and limits its systematic capabilities.

In *Drosophila melanogaster* embryos, high collagen components are essential for formulating, synthesizing, and secreting substances (Wilmes et al., 2018). In addition, high collagen components are foods believed to nurture collagen production by building amino acids such as hydroxyproline. Without collagen, the body would weaken, and the organism may experience joint pain and stiff ligaments.

Drosophila Melanogaster has already been previously used countless times to demonstrate its correlation with obesity. The organism contains tissues, organs, and system analogs related to obesity in humans. They have the capability to develop obesity when faced with a caloric overload, similar to humans (Musselman & Kühnlein, 2018). Furthermore, *Drosophila Melanogaster* has been used in applying high-sugar and high-fat diets to promote obesity and type-2 diabetes. The organism is able to experience the adverse effects of obesity

and provides testable circumstances for research. More importantly, obesity's impact leads to changes in the ECM (extracellular matrix), which is composed of many families of molecules, including collagen. With the effects of obesity on the *Drosophila Melanogaster,* anatomical changes in both diet and locomotive ability are presumable.

Diets high in fats and sugars impact the internal functioning systems within the organism's body, such as life span, locomotor activity, and carbohydrate-insulin homeostasis (Eickelberg et al., 2022). Specifically, in *Drosophila melanogaster*, the difference in diets can be used to measure the food intake of the results on a standard blue medium diet after the 5-day dietary restrictions. The capillary feeder assay measures food consumption after the diet, resulting in changes in dietary behaviors. A capillary feeder assay is used due to its variety of applications for quantifying the role of food (Diegelmann et al., 2017). With this assay in place, the role of obesity on the *Drosophila Melanogaster* can be proven through its phenotype and relative differences in food intake. Furthermore, when comparing the different test groups, the capillary feeder assay aids in reconfirming the role of obesity, as the test group may have significantly higher feeding rates.

After observing physical factors, collagen production may be tested to see if there is any correlation between excess nutritional diets that promote high sugars and fats alongside measurable collagen levels in the model organism. In this study, *Drosophila melanogaster* helps represent collagen production's impact on human bodies. Many disregard collagen's roles in their body and do not signify its importance. However, collagen helps support bodily functions, prevent rapid tumor growth, and to avoid muscle problems (Xu et al., 2019). Harmful habits like smoking and poor diets can decrease collagen cell production and internal bodily functions. Because of this, older generations may become more aware of collagen supplements since they help ease these negative symptoms. Symptoms may include papery skin, wrinkles, and even slow muscle recovery. With the use of collagen supplements, the aging process can be slowed down dramatically, along with the creation of stronger bones or skeletal structures (Reilly & Lozano, 2021).

A collagen mutated group, obesity-mutated group, and wild-type group help to best assess the relationship between overnutrition and collagen production. Mutations in collagen result in osteogenesis imperfecta, more commonly known as brittle bone disease. A reduction in collagen levels characterizes this. Using the collagen mutated group reassures results for the collagen and hydroxyproline levels in the wild-type group and obesity group, as they must surpass the collagen mutated group. The phenotype for the collagen mutated group includes abnormal size during the adult stage, lethality before reaching maturity, and heat sensitivity. An obesity-mutated group utilizes a gene mutation that enhances the ability to become obese and diminishes the feeling of fullness, causing the organism to consume larger quantities of food. With an obesity mutated test group, obesity is easily induced in the *Drosophila Melanogaster*, following the obesity-inducing diet. The phenotype for the obesity mutated group includes decreased rates of movement, abnormal development rates, and abnormal feeding behavior. Finally, a wild-type group helps to provide a negative control for the results attained in the experiment, comparing any differences. It is hypothesized that if *Drosophila melanogaster* engages in overnutrition, then changes in the extracellular matrix, particularly collagen, will lead to anatomical changes because of differences in both movement and diet.

Methods

Three different types of flies were used: wild, collagen mutant, and obesity mutant. Each condition consisted of ten flies placed on a vial. The experiment was run across five days, and the flies were fed enough food to last the week. The test subjects were transferred into the same condition vials if larvae were formed, specifically with only 10 flies per vial. The flies were maintained at twelve hours light and twelve hours dark, controlled by the lightning of the laboratory.

Diet Protocol: Wild-type Flies: *Drosophila melanogaster* are often fed solid diets mixed with water to produce moist food. The food usually contains blue food, water, and yeast. The food-to-water medium was 1:1

(using 10 ml of food, 10 ml of water, and 10 yeast balls). The food was left for 30 minutes at room temperature before feeding to flies, then kept at 4 °C when not used.

The following protocol applies for feeding 10 flies, equivalent to a 5-day period of food.

1. Use a graduated cylinder to measure 10 ml of Formula 4-24 Blue Medium Food.
2. Place the medium in pre-measured 10 ml of distilled water in a small vial, and dilute by moving the vial as much as possible without spilling the food.
3. Using a CO_2 *Drosophila melanogaster* anesthetizer, put the flies to sleep.
4. Transfer 10 flies per vial containing 10 ml of blue food, 10 ml of water, and 10 yeast balls.

Collagen & Obesity Mutant Flies: An obesity-induced diet is created by adding excess coconut oil and glucose to promote higher sugar and fat production. Therefore, Nutri agar food supplements would be used in place of the blue food medium diets.

The following protocol applies for feeding 10 flies, equivalent to a 50-day period of food.

1. Take a bag of Nutri agar and add 500 milliliters of distilled water to 78 grams of Nutri agar in a glass beaker on a hot plate at high temperatures. Mix continuously throughout the process.
2. Wait until the Nutri agar solution boils, then lower the heat level to medium-low and add 5 milliliters of preservative, 150 milliliters of coconut oil, and a glucose-water solution of 50 grams of glucose with 100 milliliters of distilled water. Mix continuously throughout the process.
3. Raise the temperature of the hot plate again back to high and wait for the Nutri agar mixture to boil again. Once the solution boils, this means that it is completely mixed all throughout the liquid. Mix continuously throughout the process.
4. Take the solution of the hot plate and lower the temperature of the hot plate to 0. Use a hot glove to remove the beaker from the hot plate. Then, slowly set it on the table.
5. Using a pipette, transport 10 milliliters of the Nutri agar solution into 73 *Drosophila melanogaster* vials.
6. Store the Nutri agar solution in the fridge to cool overnight. After waiting approximately 12 hours, the solution is ready to be used.

Preparation of Drosophila Melanogaster

1. *Drosophila melanogaster* were kept under natural light conditions in a room at 23°C while the experiment was conducted during light conditions.
2. Keep flies on the standard diet, relative
3. Use 10 flies per vial, with each mutant being contained together and divided separately.

Capillary Feeder Assay Protocol

This assay is completed after the 5-day diet to measure differences in different variability groups such as collagen, obesity, and wild-type mutant hunger levels. This assay measures the consumption of liquid food and demonstrates the differences in food consumption, if any, between the three test groups.

Use a *Drosophila melanogaster* culture plastic vial as a tube for the assay.

Anesthetize 30 *Drosophila melanogaster* and distribute them equally amongst 3 *Drosophila melanogaster* vials.

Cover the vial with 5 small layers of parafilm to prevent *Drosophila melanogaster* from escaping.

Obtain a micropipette and micropipette tip of 0-200 microliters. Make sure to mark the capillary tip accordingly to how much was filled with the sucrose-yeast solution.

Obtain a capillary tip and cut about ½ of an inch off the micropipette tip to aid in fitting the capillary tip through the micropipette.

Use a needle to make a small hole through the parafilm, and move it around to make the hole slightly larger.

Put the 45 microliter capillary tip through the micropipette and the 2 conjoined pieces through the small parafilm hole made by the needle.

Fill the capillary tip with 20 microliters of the 5 grams of yeast extract, 5 grams of sucrose, 1 microgram of erioglaucine disodium salt, and 100 milliliters of distilled water solution using gel 0-20 microliter gel loading tips.

Place parafilm on top of the capillary tip so the liquid solution does not slowly fall out.

After 2 days, view the solution concentration left and measure the solution consumed. Repeat this process for each *Drosophila melanogaster* vial being used.

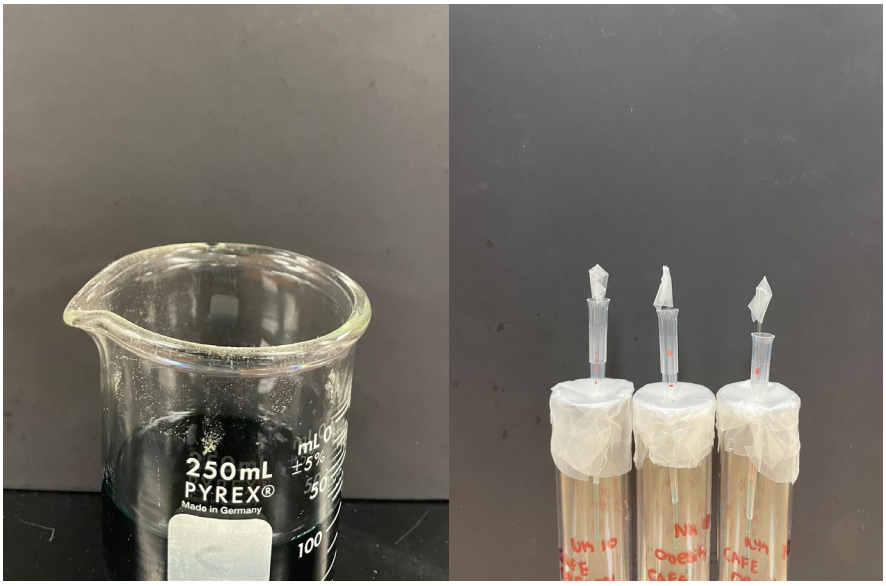

Figure 2. From Left to Right, The Capillary-filled Liquid Food Makeup, Along With Setups for the CAFE Assay

Negative Geotaxis Assay Protocol

A negative geotaxis assay is used to measure the climbing ability of the flies. This can be an alternative to an imaging assay because it provides data on the test subjects' speed, durability, and even strength. A total of 4 trials of 3 distinct centrifuge tubes containing 10 flies in the same mutant group were used to create 12 total repetitions.

1. Obtain a centrifuge tube.
2. Mark 8 centimeters on the centrifuge tube.
3. Anesthetize the flies using CO2.
4. Transfer the test group of 10 flies into the centrifuge tube.
5. Screw the centrifuge cap halfway to let the *Drosophila melanogaster* breathe.
6. Allow the flies to rest for 20 minutes before experimentation.
7. Tap the centrifuge tube 10 times to bring the *Drosophila melanogaster* to the bottom of the tube (the cap area).
8. Start recording and observe how many flies pass the 8-centimeter mark after exactly 10 seconds.
9. Repeat for each centrifuge.

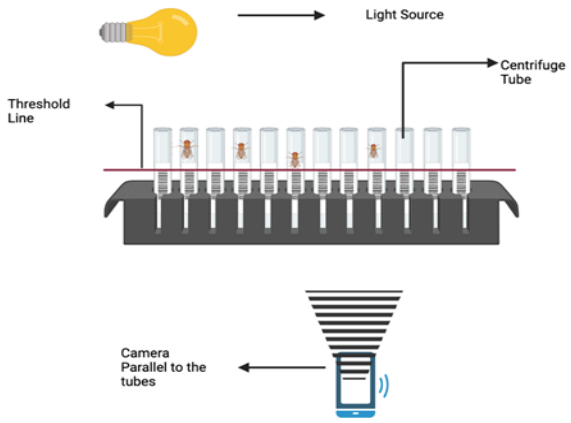

Figure 3. The Setup of Negative Geotaxis Assay [Created in BioRender]

Hydroxyproline Assay Protocol

A hydroxyproline assay is used to measure hydroxyproline and collagen concentrations within each fly group. Hydroxyproline is an important building amino acid for collagen, so collagen concentrations are proportional to hydroxyproline concentrations.

1. Standards Prep Prepare 50 micrograms/mL Hydroxyproline Standard by mixing 20 microliters of 1 mg/mL Hydroxyproline Standard and 380 microliters of distilled water. Next, prepare standards in 1.5-mL centrifuge tubes as described in the table below. The following standards include 100 microliters of 50 microgram/mL Standard with 0 microliters of water and 50 micrograms/mL of Hydroxyproline, 60 microliters of 50 microgram/mL Standard with 40 microliters of water and 30 micrograms/mL of Hydroxyproline, 30 microliters of 50 micrograms/mL Standard with 70 microliters of water and 15 micrograms/mL of Hydroxyproline, and 0 microliters of 50 microgram/mL Standard with 100 microliters of water and 0 micrograms/mL of Hydroxyproline.
2. Transfer 20 microliter standards and samples into separate wells of a clear, flat-bottom 96-well plate.
3. Mix 8 microliters of Reagent A with 90 microliters of Oxidation Buffer for each well. Add 90 microliters of this mix to each well. Tap the plate to mix and incubate for 10 minutes at room temperature.
4. Add 90 microliters of Reagent B to all wells. When Reagent B is added, the wells will become turbid, and one must pipette up and down until the turbidity dissipates.
5. Incubate for 90 invites at 37°C in a plate reader or incubator.
6. Read ODs at A = 560 nm. Use OD at 90-minute time points for analysis.

Calculation: Subtract the blank value (#4) from the standard values and plot the △OD against standard concentrations. Determine the slope and calculate the hydroxyproline concentration of the sample.

$$[\text{Hydroxyproline}] = \frac{OD_{Sample} - OD_{Blank}}{Slope\ (\mu g/mL^{-1})} \times\ n\ (\mu g/mL)$$

where OD Sample and OD Blank are OD readings of the Sample and Blank, respectively. n is the sample dilution factor. Note: The dilution factor n for the hydrolysis protocol is 6 (50 microliter sample + 50 microliter 10N NaOH + 50 microliter 10N HCl + 150 microliter distilled water).

Conversions: 50 micrograms/mL equals 5 mg/dL or 50 ppm. Hydroxyproline constitutes 13% of total collagen weight on average. 1 microgram/mL Hydroxyproline is equivalent to 1/0.13 or 7.69 microgram/mL collagen.

Results

A Capillary Feeder Assay, Hydroxyproline Assay, and Negative Geotaxis Assay are used to gather any possible data. This data can be used to compare and contrast the hypothesis. Charts and graphs will be used to record data from each assay, utilizing the ability to compare and contrast different results between assays.

Table 1. The Composition of the Wild-Type Diet for 10 *Drosophila Melanogaster*

Ingredients	Measurements
Formula 4-24 Medium Blue Food	10 ml
Water	10 ml
Yeast	10 balls

Table 2. The Composition of the Obesity-Inducing Diet for 10 *Drosophila Melanogaster*

Ingredients	Measurements
Nutri Agar Powder	1.07 g
Agar Preservative	0.068 ml
Distilled Water	8.2 ml
Coconut Oil	2.05 ml
Glucose	0.68g

Diet compositions: The wild-type diet is traditionally composed of *Drosophila melanogaster* blue food with water and yeast, providing a moist and ideal environment. However, to influence and induce obesity with a high-fat and high-sugar diet, a Nutri agar composition was created. This diet increases sugars and fats, making the obesity factor needed to test the subjects in this experiment. Using coconut oil and glucose created an environment too liquified to use traditional Formula 4-24 Blue Medium, so a Nutri agar powder and preservative were used.

Negative Geotaxis Assay

Table 3. Results from the Negative Geotaxis Assay, Wild-type Condition

Trial 1			
	Centrifuge 1	Centrifuge 2	Centrifuge 3
Passed The line	2	5	3
Did Not pass The Line	8	5	7
Trial 2			
Passed the Line	5	2	1
Did not pass the Line	5	8	9
Trial 3			
Passed the Line	4	6	5
Did not pass the line	6	4	5
Trial 4			
Passed the line	3	5	3
Did not pass the line	7	5	7
Average	3.5	4.5	3

Four trials of three centrifuges were run throughout the experiment, attaining 12 different samples. About 36.6% of flies passed the 8-centimeter line marking provided. A total of 44 out of 120 flies were able to climb over the line. These results will be used as a control to compare the results from Wild-type flies with obesity and collagen mutants. The Wild-type flies had the highest locomotive ability rate compared to the other fly groups because their passing rate was significantly higher. Therefore, the negative geotaxis assay results with the wild-type group acted as a standard measuring point for the other two fly groups.

Table 4. Results from the Negative Geotaxis Assay, Collagen Mutant Condition

Trial 1			
	Centrifuge 1	Centrifuge 2	Centrifuge 3
Passed The line	1	3	0
Did Not pass The Line	9	7	10

Trial 2			
Passed the Line	0	2	2
Did not pass the Line	10	8	8
Trial 3			
Passed the Line	2	1	3
Did not pass the line	8	9	7
Trial 4			
Passed the line	2	0	4
Did not pass the line	8	10	6
Average	1.25	1.5	2.25

Regarding the obesity mutant in the Negative Geotaxis assay, four trials of three centrifuges were run, gaining 12 varied samples. The phenotype for the obesity mutated group includes decreased rates of movement and abnormal feeding behavior. About 16.7% of flies passed the 8-centimeter line marking. A total of 20 out of 120 flies were able to climb over the line. There is a vast difference between the Wild-type and obesity mutant, providing a 19.9% decrease rate in locomotive ability. This shows how the Nutri agar diet induced obesity, leading to slower and weaker external bodily functions. Based on the data presented, the average pass rate dropped while the did not pass rate vastly increased.

Table 5. Results from the Negative Geotaxis Assay, Collagen Mutant Condition

Trial 1			
	Centrifuge 1	Centrifuge 2	Centrifuge 3
Passed The line	2	5	4
Did Not pass The Line	8	5	6
Trial 2			
Passed the Line	1	6	3
Did not pass the Line	9	4	7
Trial 3			
Passed the Line	3	3	2
Did not pass the line	7	7	8

Trial 4			
Passed the line	2	3	2
Did not pass the line	8	7	8
Average	2	4.25	2.75

During the collagen mutant Negative Geotaxis assay, four trials were performed with three separate centrifuge groups, attaining 12 different samples. The phenotype for the collagen mutated group includes abnormal size during the adult stage and lethality before reaching maturity. About 30% of test subjects passed the 8-centimeter line marking. A total of 36 out of 120 flies were able to climb over the line. Comparing these results to the Wild-type condition, the average decreased by 6.6%. Furthermore, the Nutri agar diet affected the locomotive ability of the collagen-mutated test subjects. It slowed their climbing ability, but also external bodily functions overall. The pass rate decreased while the did not pass rate adequately increased, showing a variation when compared to the wild-type control group.

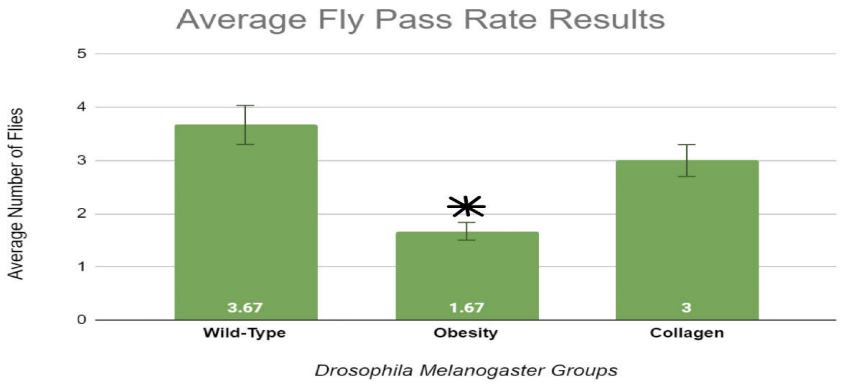

Figure 4. The Average Passing Rate Compared Through Each Test Group

This graph shows the average passing rate for each test group. After their 5-day diet, the wild-type group had the highest locomotive rate, the mutated collagen group had a mid-range rate, and the obesity mutated group, after the 5-day diet, had the lowest locomotive ability rate. The locomotive ability reconfirmed the phenotypes for the obesity-induced diet on the obesity-mutated flies as decreased neural activity occurred and lower pass rates were experienced; this led to a lower locomotive ability rate.

Table 6. The p-value When Comparing the Wild-type Group and Obesity Group in the Negative Geotaxis Assay

Negative Geotaxis Statistical Analysis	
Drosophila Melanogaster Groups	p-value
WTvObesity	0.01997877941

Since the p-value is less than 0.05 ($p<0.05$), there is a significant difference in the locomotive ability between the Wild-type and obesity-mutated group, this reconfirms the phenotype for the obesity mutant, as they have significantly slower movement rates compared to the wild-type flies. Furthermore, it signifies that the obesity-induced *Drosophila Melanogaster* experienced decreased neural activity, which is critical in movement and suppression of neural autophagy, along with reduced metabolic rates in areas such as climbing. Due to the High-Sugar and High-Fat diets, the obesity-induced flies reached modifications in the central nervous system, creating less movement.

Capillary Feeder Assay

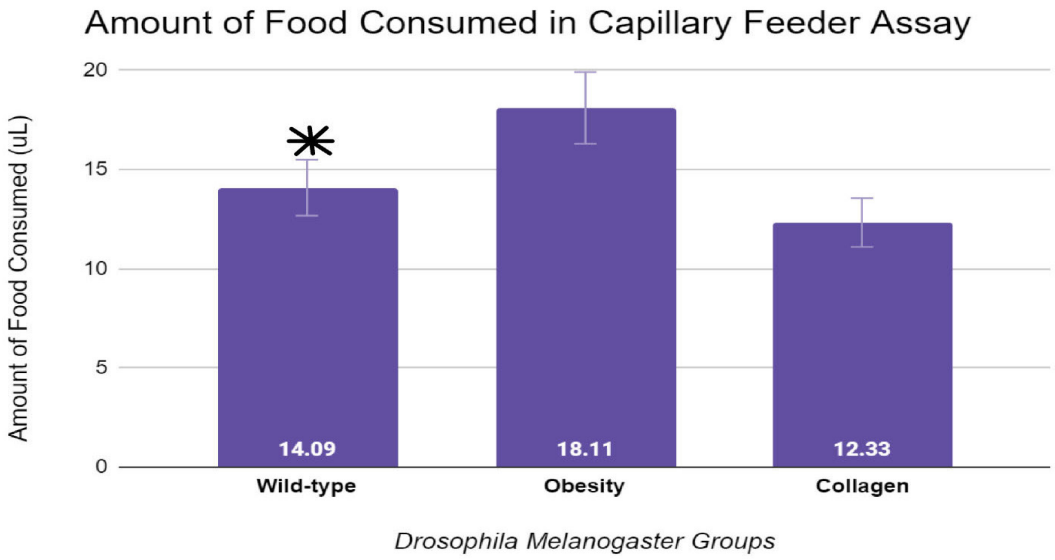

Figure 5. The Average Food Consumption Within Each Fly Group

This graph shows the average amount of food consumed by each fly group in microliters (uL). The feeding patterns for all fly groups varied. The collagen-mutated group consumed the least, the wild-type group stayed in the middle, and the obesity-mutated group consumed the most. The consumption of food was measured over two days. This also correlates to the fact that the obesity-induced *Drosophila Melanogaster* experienced higher feeding rates and inaccurate hunger and satiety perceptions, which control the ability of a fly to feel full. Due to the higher levels of food intake, decreased locomotive activity occurred. Because of the High-Sugar and High-Fat diets, the flies experienced anatomical changes regarding diet and food consumption levels.

Table 7. The p-value When Comparing the Wild-type Group and Obesity Group in the Capillary Feeder Assay

Capillary Feeder Statistical Analysis	
Drosophila Melanogaster Groups	p-value
WTvObesity	0.02277033472

Since the p-value is less than 0.05 ($p<0.05$), there is a significant difference in the amount of food consumed between the wild-type and obesity-mutated group. This reconfirms the phenotype for the obesity mutant, as they have higher feeding rates than wild-type flies.

Hydroxyproline Assay & Collagen Concentrations

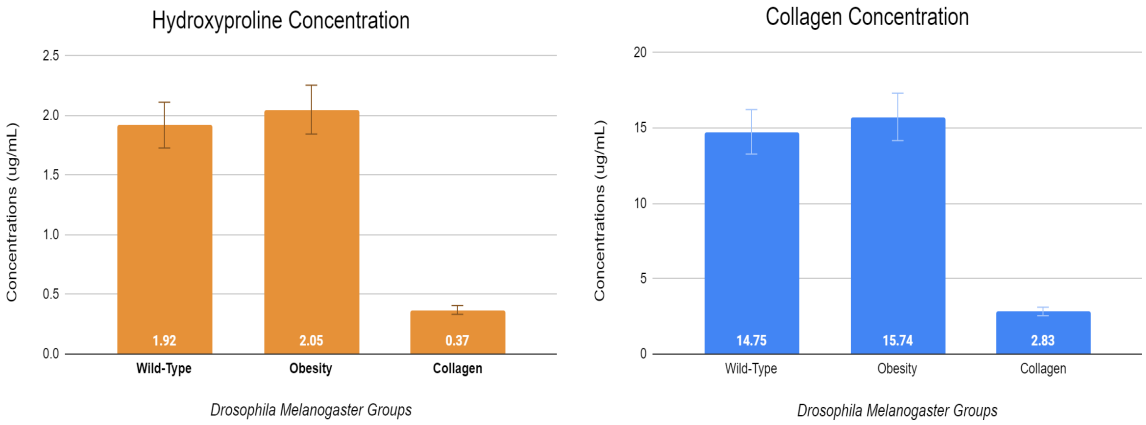

Figure 6. From Left to Right, the Average Hydroxyproline and Collagen Concentrations Within Each Fly Group

The graphs show the average hydroxyproline levels of each test group, the obesity-mutated group had the highest, the collagen-mutated group had the lowest, the wild-type group in the middle, High-Sugar and High-Fat diets slightly increased hydroxyproline, but no significant change, adipose tissue also slightly increased, 4 trials completed. Since hydroxyproline is the building amino acid for collagen, the collagen concentration is proportional to the building block. Results are comparable.

Table 5. The p-value When Comparing the Obesity Mutated Group and the Collagen Mutated Group, Along with the Wild-type Group and the Obesity Mutated Group in the Hydroxyproline Assay

Hydroxyproline & Collagen Statistical Analysis	
Drosophila Melanogaster Groups	p-value
WTvObesity	0.7711387647

The p-value greater than 0.05, no significant difference in hydroxyproline concentrations between wild-type and obesity-mutated groups, obesity-induced flies do not experience significant effects on hydroxyproline levels, slight differences correlated with anatomical differences in movement and diet.

Discussion

The original hypothesis is supported.

Both movement and diet can influence anatomical changes.

The obesity group was on an obesity-inducing diet which caused some changes. Since the negative geotaxis assay determined that the obesity group had less movement, this was related to higher levels of food intake and decreased neural activity due to the high-fat and high-sugar diet.

Regarding HFD (high-fat diet) and HSD (high-sugar diet) specifically, they suppressed neuronal autophagy, created inaccurate hunger and satiety perceptions, and enhanced the adipose area, which is also known to have the most collagen concentration in flies.

Collagen deposition was increased because of the HFD and HSD.

The following anatomical shape changes are not the same:

- Decreased neural activity (less movement, behavioral)
- Higher feeding rates (capillary feeder assay, behavioral)
- Enhanced adipose tissue (collagen-heavy tissue areas, anatomy)
- Suppressed neuronal autophagy (physiology)
- Decreased metabolic rates (less locomotive ability, behavioral)
- Modifications in the central nervous system (less movement, negative geotaxis assay, behavioral)

Conclusion

Based on the data and observations presented, when *Drosophila Melanogaster* is induced with overnutrition, collagen production levels are increased due to larger quantities of adipose tissue in the body. Overnutrition leads to increased food consumption rates and decreased neural activity, leading to reduced locomotive abilities in *Drosophila melanogaster* physiology. Anatomical differences in movement and diet were measured due to the High-Sugar and High-Fat diets. With an obesity-inducing diet in place, decreased metabolic rates occurred, neural autophagy was suppressed, inaccurate perceptions of hunger and satiety were created, and both collagen levels and adipose tissue were enhanced slightly. Additionally, modifications in the nervous system decreased movement rates in *Drosophila melanogaster.*

To find and analyze research in the future, look at obesity's direct correlation to collagen production and see their relationship with humans or other model organisms. Perhaps, in mammals like mice, results may differ, showing another view of this experiment. Furthermore, more assays can be done to testify to obesity's connection to collagen-like analyzing phenotypes or certain production of cells that make up the macromolecules being experimented upon.

Limitations

Drosophila melanogaster is still an animal model, so it can not directly represent humans for this study. There are other factors not accounted for, such as sex and weight, that may influence the results of certain assays and data points. Furthermore, certain areas are more abundant in collagen levels; however, in the experiment, the whole organism was used, which can lead to lower collagen concentrations and ratios since collagen is not an extremely abundant protein.

Acknowledgments

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