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Treating Alzheimer's: Finding the Relationship Between a High-Sugar Diet and the Severity of Alzheimer's

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ABSTRACT

Alzheimer's disease (AD) affects millions of Americans, characterized by progressive cognitive decline and memory loss. Despite over a century of research since its identification, gaps remain in understanding AD's causes and effective treatments. Recent studies suggest dietary factors influence AD progression, but the impact of high-sugar diets is underexplored. This study investigates how a high-sugar diet affects Alzheimer's severity using Drosophila melanogaster, a model organism with 75% of human genes. The study addresses whether high-sugar diets impact AD severity based on sex-specific differences in climbing patterns and eye development. The null hypothesis, stating that a high-sugar diet has no effect on AD severity, was rejected, with p-values of .001757 for climbing assays and .000067 for eye development, both below the significance threshold of .05. This indicates a significant impact of high-sugar diets on AD severity. Sex-specific results showed that female Drosophila exhibited greater resilience against AD-related neurodegeneration compared to males, contrary to some human studies suggesting more severe outcomes in women. This may be linked to biological differences, such as hormonal influences or metabolic responses. Female flies performed better in climbing assays and exhibited slightly better eye development under a high-sugar diet compared to males. These findings underscore the importance of considering sex-specific factors in AD research and potential dietary interventions. Future research should explore these effects in more complex organisms and investigate how various dietary components influence AD progression and protein aggregation, aiming to refine therapeutic strategies and personalized medicine approaches.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that "destroys neurons and their connections in parts of the brain involved in memory" and affects about six million Americans (National Institute on Aging, 2017). It is characterized by progressive cognitive/locomotive decline and memory loss. While extensive research has been conducted to better understand the disease, significant gaps in our knowledge persist. These gaps are revealed throughout. Alzheimer's research, from risk factors to effective treatments and prevention strategies.

In 1906, neuroanatomist Alois Alzheimer, reported "a peculiar severe disease process of the cerebral cortex" (Hippius & Neundörfer, 2003). First identified over a century ago, Alzheimer's has remained an enigmatic condition, defying comprehensive understanding and effective treatment. Neurodegenerative diseases, such as Alzheimer's, have unknown causes.

Alzheimer's has garnered increasing attention and significance within the medical field. According to the Center for Disease Control (CDC), "scientists do not yet fully understand what causes Alzheimer's" (CDC, 2023). Its impact on millions of individuals and families, coupled with the growing aging population, has driven research for innovative strategies for diagnosis, treatment, and prevention. This pursuit of understanding Alzheimer's has not only deepened knowledge of the human brain but has redefined the boundaries of medical science, offering both hope for those affected and insights for the broader field of medicine, especially in the 21st

century.

Recently, research has studied other factors, like diet. However, information about how certain diets affect Alzheimer's risk factors has not been researched. While research has shown that high-fat diets have an impact on brain degeneration, high-sugar diets have not been studied. In order to see this degradation, my research must be able to analyze brain degeneration. Drosophila melanogaster (fruit flies) are good model organisms for studying the loss of cognitive and locomotive functions in AD, therefore, are excellent tools for studying AD, since they share approximately 75% of human genes (Kasanin et al., 2022). By feeding Drosophila a high-sugar diet, I can determine neurodegeneration in Alzheimer's.

The medical field is expanding quickly and researchers need to be able to find proper treatments for neurodegenerative diseases. With dietary intake and sex-specific differences, the medical field needs to understand the severity of Alzheimer's. Therefore, my study answers the question: What are the effects of a high-sugar diet on the severity of Alzheimer's based on Drosophila melanogaster sex-specific climbing patterns and eye development?

Review of Literature

Research on Alzheimer's Disease

Alzheimer's Disease (AD) is a type of dementia that negatively affects cognitive and locomotive functions in the brain (Alzheimer's Association, 2023). The main issues pervading the research of AD are that researchers are not able to fully understand the cause and treatment to cure AD. AD is a major challenge for global health and social care, and current understanding of AD pathogenesis is limited (Sun et al., 2018). As researchers continue to find optimal treatment for this illness, AD treatment remains unknown to the medical field.

Throughout AD research, a core factor in identifying AD has been its progression over time. AD progresses slowly in three stages: early, middle, and late (Alzheimer's Association, 2020). In the early stage of Alzheimer's, the individual may function independently. Their motor functions and neuron development are mostly intact. In the middle stage, the symptoms are more pronounced. There is a greater loss of motor function. The damage to nerve cells in the brain makes it difficult for the person to express thoughts and perform routine tasks without assistance. In the late stage, the individual loses the ability to respond to his/her environment and control movement (Alzheimer's Association, 2020). However, there have been ways to detect the progression of AD. In fact, Sun and others (2018) say that tools, such as "amyloid and tau PET imaging, clinical trials targeting amyloid-beta (Aβ), tau, and neurotransmitters" are able to provide new perspectives on the efforts to help treat AD. However, while there are tools that can help determine the progression and severity of this disease, not enough research has been done to create a perfect treatment for avoiding the progression of AD.

Differences Between Male and Female Brains

Although male and female anatomy have similarities, they differ in terms of neurological functions, and the factors between male and females are not understood. Pike (2017) found that both estrogens and androgens exert protective actions in the adult brain that increase neural functioning and resilience, specifically in neuropathology. Estrogen, which is a female hormone, is associated with increased AD risk, however, androgens, which are male hormones, do not exhibit age-related reduction and are not significantly associated with AD risk. However, depletions of testosterone in the brain predict enhanced vulnerability to AD. Clearly, AD affects males and females differently.

Similarly, Budson (2022) agrees that there are differences between male and female brains in terms of AD that haven't been properly looked at. Female neurological function and enzyme production is very different from males. Therefore, females are more likely to develop Alzheimer's due to their life longevity compared to men. In fact, around twice as many women have Alzheimer's compared to men (Alzheimer's Society, 2023). Because of the stronger

female immune system, women's brains end up having more plaque accumulation. These plaques build up over time and cause Alzheimer's (Budson, 2022).

Others found the same results. Beam et al (2018), followed 16,926 people in Sweden and found that, around age 80, women were more likely to be diagnosed with Alzheimer's than men of the same age. Similarly, Liu et al (2019), in Taiwan, found that one's chances of developing Alzheimer's was greater in women compared to men. Finally, H & Alvarez (2017) examined the incidence of Alzheimer's in Europe. They found that approximately 13 women out of 1,000 developed Alzheimer's each year, compared to only seven men. However, more evidence is needed to fully determine the difference between male and female AD.

A Diet's Effect on the Brain

The consumption of different proteins through food can have a direct or indirect impact on the brain's cognitive and locomotive functions. Foods that contain sugar and fat can lead to severe impacts on the brain, especially in regards to Alzheimer's. Liu et al (2022) found that "excessive total sugar intake was significantly associated with AD risk in women". While this suggests that sugar intake increases the risk of AD, it only considers female brain rather than male brain functions. Similarly, the University of Michigan claimed that sugar compounds impact brain health in *Drosophila* (Sherburne, 2019). The researchers found that the flies 'metabolic profiles change rapidly during the quick transition from hunger to satiety, with the flies 'brains showing a larger change than their phenotypic features. In particular, the high-sugar diet lowered the levels of the brain metabolites N-acetyl aspartate (NAA) and kynurenine. However, while they had suggested that high-sugar diets lower levels of brain metabolites, it doesn't specifically study Alzheimer's.

In addition, Moreira (2013) agrees with both of these studies and found that high-sugar diets are known to promote weight gain and insulin resistance predisposing to Type 2 Diabetes (T2D), which has shown to be a risk factor for AD. Both shared similar demographic profiles, risk factors, and clinical and biochemical features, such as insulin resistance. She found that dietary changes can significantly reduce the risk of T2D, thereby increasing life longevity. However, she doesn't look at the direct impact of the high-sugar diet on the brain. Robison et al (2020) disagree and found that a high-fat (HF) diet resulted in the "greatest weight gain, adiposity, and glucose intolerance in 3xTg-AD females," which was associated with markedly increased hypothalamic expression in hypothalamic nuclei that regulate energy balance.

In contrast, HF diet increased diabetes markers and systemic inflammation preferentially in AD males but did not exacerbate hypothalamic inflammation. However, this is only found for a high-fat diet. The data in these studies presented further weaknesses in the direct effects of a high-sugar diet on the severity of Alzheimer's.

Drosophila melanogaster as a Good Model Organism

Drosophila are good model organisms for studying Alzheimer's in cognitive and locomotive functions. *Drosophila* are excellent tools for studying the neurological effects of AD, since they share approximately 75% of human genes (Kasanin et al., 2022). In addition, neurodegeneration in *Drosophila* manifests in phenotypic changes. *Drosophila* is a great model organism because of their "short generation times and facile genetic analysis" (Kasanin et al., 2022). They looked at three different models of AD in *Drosophila*, the C99 model, Aβ42 model, and the TAU (Tubulin-Associated Unit) model. My study used the TAU model since it gives a clearer distinction of the impacts of AD in terms of locomotive functions and eye development.

Kasanin, again, shows that in *Drosophila*, pan-neuronal expression of TAU can lead to locomotor deficits and neurodegeneration of brain regions. Various studies have used *Drosophila* as a model to investigate TAU in AD.

Similarly, Yamaguchi (2018) found that *Drosophila* are commonly used in medical experiments. Compared to mice, working with *Drosophila* requires simpler procedures, however, have not been used for dietary impacts.

Yokoyama (2022) suggested that no Alzheimer's mouse model has been created to mimic the TAU deposition observed in sporadic AD patients. Interestingly, Yokoyama found that cats, dogs, and nonhuman primates all naturally develop age-dependent Alzheimer's-like brain pathologies. While they believe that mouse models of Alzheimer's are more dependable, other researchers, such as White (2021) disagree. White studies behavioral changes, including aggression and depression, in *Drosophila*. Using *Drosophila*, White investigates the role of TAU proteins in the development of neurodegenerative diseases related to traumatic brain injury. Clearly, neurodegeneration in *Drosophila* shows a strong correlation with the expression and spread of tau throughout the brain's circuitry (White, 2021).

Similarly, in reference to Kasanin el at (2022), this means that there could be a strong correlation between a high-sugar diet and the expression of AD, however this has not been studied. The use of *Drosophila* has been valuable to Alzheimer's research, which allows my study to accurately compare the data.

Drosophila melanogaster Climbing Patterns

The methods of assessing locomotive functions in *Drosophila* consist of a negative geotaxis assay (climbing assay), a crawling assay, and a flight assay. However, the crawling assay is only used for larvae, which wouldn't be applicable for my study. My study uses a climbing assay as it is most effective in providing accurate and analytical results quickly. Madabattula et al (2015) found, through the analysis of climbing patterns, an increasing number of genes have been identified to play a role in locomotion. However, despite identifying these genes, it is unknown how these genes contribute to normal locomotive functioning. Therefore, Madabattula et al (2015) experimented to find a reliable assay, which utilizes model organisms to "elucidate the role of these genes in order to identify novel targets of therapeutic interest.".

Similar to other research, Madabattula et al (2015) created a sensitized version of a negative geotaxis assay, also known as a climbing assay, that allows for the detection of milder defects. The assay is cost effective and does not require extensive training to obtain highly reproducible results. This allows it to be the best technique for screening locomotive defects due to neurodegenerative diseases, such as AD. My study used Madabattula's methods, however, *Drosophila* fed a high-sugar diet rather than a candidate drug. Similarly, Manjila (2018) agrees, however, she analyzes a flight assay. She suggests that motor control is required for flying, walking, feeding and mating. Like my study, any perturbation to the sensory input or malfunctioning of neural connections to the motor output can result in motor defects. While she determines that results from flight assays are accurate, she claims that a climbing assay delivers quicker and *more* accurate results as compared to flight assay.

However, while these researchers agree with one another, Nichols (2012) disagrees and claims that a crawling assay in the larval stage of *Drosophila* is more accurate when finding larval locomotion. While this could be true, my study is being done with adult flies, so a crawling assay is not applicable. He found that larval crawling assays become more applicable if expression or abolition of a gene causes lethality in pupal or adult stages since these flies do not survive to adulthood. However, while a crawling assay has been used to look at the effect of drugs, like in Madabattula's study, my study uses it to quantify the effects of a high-sugar diet in *Drosophila* with AD.

Drosophila melanogaster Eye Development

Several researchers have looked at studying eye development in *Drosophila*, shedding light on fundamental biological processes. Baker et al (2014) explains the critical role of transcription factors in the patterning of *Drosophila* eyes. Similarly, my study builds upon looking at specific patterns within *Drosophila* eyes to see whether a high-sugar diet impacted the genetic proteins within them. Furthermore, Cagan (2009) investigated the impact of specific genetic mutations in the differentiation of photoreceptor cells, uncovering crucial insights into the molecular mechanisms underpinning eye morphogenesis. Additionally, similar to Cagan and Baker, recent work by Friedrich (2003) has extended our understanding, emphasizing the significance of gene identification in coordinating cell proliferation

during fruit fly eye development. Together, these studies contribute to a comprehensive picture of the genetic and molecular identities that unfold within the eyes of *Drosophila*, providing valuable knowledge with potential implications for broader insights into developmental biology.

The Gap

Throughout this research, we can see that dietary choices have a significant impact on the neurodegeneration in the brain. However, there has not been significant research done to find the direct impacts. With dietary choices, much of the research has shown an indirect relation to AD neurodegeneration. Moreira (2013) suggests that an excessive high-sugar diet has an indirect impact on AD, however, it does not directly show results. In addition, the research that has been done on AD doesn't necessarily compare females and males either. Budson (2020) found differences in amyloid plaques between women, however he did not look at men. This study addresses the gaps found in the research using the research question: What are the effects of a high-sugar diet on the severity of Alzheimer's based on *Drosophila melanogaster* sex-specific climbing patterns and eye development?

Methods

To analyze the relationship between development, Alzheimer's, and a high-sugar diet in *Drosophila*, the experimental approach has many key strengths. An experimental approach determines the true relationship between high-sugar and the severity of Alzheimer's through *Drosophila*. My study explains the experimental causal relationship between a high-sugar diet and severity of Alzheimer's through *Drosophila* based on locomotion and eye development. Since the effects in my study are only caused by high-sugar, the experimental design allows me to apply my findings to the human consumption of a high-sugar diet and suggest dietary changes that help prevent Alzheimer's progression. An experimental design is the optimal design to explain the relationship between these two variables to treat *Drosophila* and measure the effects of the diet. With my research question, the 'explaining 'approach allows me to make a connection between a high-sugar diet's impact on the severity of Alzheimer's. The purpose of this study is to explain the causal relationship between dietary intake and Alzheimer's degeneration.

Experimental Method

The experimental research method stands out as a superior approach for measuring neural functions in *Drosophila* compared to the causal-comparative research method. In experimental research, the study has precise control over variables, allowing for the systematic manipulation of factors, such as diet or genetic traits, that may influence climbing behavior (Manjila, 2018). By using the experimental design for my study, I am able to test the severity of Alzheimer's using variables that I can manipulate. Other studies, such as Manjila (2018) and Madabattula (2015), also use these methods when finding changes in locomotive functions. It enables isolation of specific causal relationships in the results rather than in the entire experiment. Contrastingly, causal-comparative research relies on a non-experimental design set-up, meaning that I would not be able to directly manipulate variables. This limitation hinders the establishment of a cause-and-effect relationship, making it challenging to discern whether neurodegeneration is influenced by the intended factors. The experimental method's capacity for controlled manipulation makes it a more potent tool for researching the nuanced climbing behavior of *Drosophila*, providing a clearer understanding of the variables influencing their motor capabilities. This experimental research method promotes the strengths of the approach, design, and method of this study.

Mixed Methods

This study relies on mixed methods. Bringing qualitative data and quantitative data together allows the data to align with the research question and purpose of this study. It offers a comprehensive approach for analyzing climbing patterns in *Drosophila* compared to relying solely on qualitative data. While quantitative data provides valuable numerical metrics regarding climbing patterns, it lacks the depth needed to fully grasp the nuances of behavior. With qualitative and quantitative data, my study observes visual and numerical abnormalities in Alzheimer's modeled within *Drosophila*. This method is pertinent in the context of studying climbing patterns, where factors beyond distance climbed may influence the overall assignment. Similarly, Aggarwal et al (2019) suggested mixed methods as an optimal method for climbing assays for their study on Parkinson's. Like my research, their qualitative insights uncovered unforeseen behavioral traits that quantitative data alone may overlook. For this study, I measured the pattern of their climbing qualitatively; whether it is 'zig-zag 'or 'fairly straight'. The qualitative and quantitative methods allow for the detection of milder defects earlier and the ability to evaluate these defects over time (Madabattula, 2015). Then, I used a correlation statistical test to correlate both variables. Thus, a mixed methods approach enhances the interpretability of climbing patterns in *Drosophila*.

Experimental Setup

One of the foundational pillars of this study lies in the precision of our genetic strain selection. The process of choosing *Drosophila* strains expressing Alzheimer's traits ensures the specificity and accuracy of the experimental groups. This precision not only enhances the relevance of the finding to Alzheimer's, but also establishes a robust genetic framework for investigating the impact of dietary factors. A hallmark of this experimental design is the comprehensive treatment of dietary variables. The formulation and administration of a high-sugar diet, contrasted with a carefully defined control diet, reflects the causal results embedded in the approach. This dietary manipulation allows for a nuanced comparison, minimizing confounding factors.

Conducting the Experiment

For this study, I fed a high-sugar diet to *Drosophila* and observed the impacts it had on locomotive functions. I kept surroundings around standard conditions which are 22-25 degrees Celsius. Every week, I switched the fruit flies to new vials to maintain the freshness in the fruit fly food, as researchers recommend switching vials between every 3 to 14 days (Carolina Biological Supply Company, n.d; Westfall et al., 2018). For the high-sugar diet, I mixed dry potato flakes with 50 mL of sucrose solution (sugar mixed with distilled water) and kept it at the bottom of their vials. I created a high-sugar diet with 10% more sucrose by mixing 1 gram of dry potato flakes and 50 mL of sucrose solution. I separated out groups of male and female flies and kept them in their vials. I had a control group for each of the genders. After allowing the flies to feed for two weeks, I transferred them into a separate test tube using a small brush and a foam stopper to close the vials. Then, I tapped them to the bottom of the vial. Once they were at the bottom of the vial, I set up a camera to record and analyze the abnormalities in their climbing patterns, such as shortened climbing distances. While the camera recorded, I timed how long it took for 30 randomly chosen flies from the vials to climb past 7 cm in 7 seconds. Then, I used a microscope to look at the eye development. After getting this data, I repeated the process with the control groups and compared the two sets of data.

Ethical Considerations and Limitations

Ethical considerations and the commitment to humane treatment are integral strengths that underpin the entire study. My emphasis on responsible research practices not only aligns with ethical standards but also contributes to the credibility of my study by ensuring the well-being of the experimental subjects. Feeding a high-sugar diet to *Drosophila*

has limitations and weaknesses. Excessive sugar intake could lead to altered physiological responses, affecting the relevance of findings. Feeding *Drosophila* a high-sugar diet could result in developed obesity and type 2 diabetes-like pathophysiology, which could impact the results (Murashov et al., 2021). High-sugar diets could induce metabolic changes that aren't representative of natural conditions, which may impact the study's external validity.

Additionally, the focus on sugar alone may overlook interactions with influences. This could potentially oversimplify the nature of dietary influences on *Drosophila*. Considering this, I conducted research on the proper sucrose concentrations to avoid inducing another metabolic change or sugar-related disorder.

Results

As discussed in the methods, the climbing patterns and eye tissue development was tested through male and female flies. To reiterate, my research question is: What are the effects of a high-sugar diet on the severity of Alzheimer's based on *Drosophila melanogaster* sex-specific climbing patterns and eye development? My data fills the gap in Alzheimer's therapeutic treatment studies for those suffering.

Null Hypothesis: A high-sugar diet has no impact on the severity of Alzheimer's in *Drosophila*.

Alternate Hypothesis: A high-sugar diet increases the severity of Alzheimer's in *Drosophila*.

Climbing Patterns

To investigate the effects of a high-sugar diet on *Drosophila*, a climbing assay analyzing climbing patterns serves as a valuable tool for assessing the data. A climbing assay is most effective in providing accurate and analytical results quickly (Madabattula et al., 2015). For *Drosophila*, the average climbing distance within 7 seconds is 7 cm; a rate of 1 cm/s (Nichols et al., 2012). After dividing the flies into control and experimental groups and administering their respective diets, I conducted climbing assays and recorded their climbing behaviors.

Eye Development

As previously discussed, the eye tissue development of flies also serves as a valuable tool for assessing the degradation of Alzheimer's peptides in the brain. As "one of the most intensively studied organs in *Drosophila*," this experiment uses eye development as a main factor (Baker et al., 2014). For measuring eye development, I used a microscope to take pictures of the flies 'eye tissue to look for abnormal texture. Then, I used a program to take the area covered by abnormal texture and converted it into a percentage.

Fly Strains

For this experiment, I used seven different fruit fly strains. The use of multiple strains allows me to see the effects of a high-sugar diet on multiple genetic modifications. These are the seven different types:

1. Oregon-R Wildtype
2. GMR-hTau
3. GMR-hTau-hGSK3β
4. hMAPT (Tau) Wildtype
5. hMAPT-4Q
6. hMAPT-4R

Significant Results

Table 1. Qualitative analysis of climbing patterns in male *Drosophila*

Male Fly Strains	Treatment	Average climbing pattern
Oregon-R Wildtype	Normal food	straight; slow
Oregon-R Wildtype	High-sugar Diet	fairly straight; fast
GMR-hTau	Normal food	straight; slow
GMR-hTau	High-sugar Diet	zig-zag; fast
GMR-hTau-hGSK3β	Normal food	fairly straight; slow
GMR-hTau-hGSK3β	High-sugar Diet	straight; fast
hMAPT (Tau) Wildtype	Normal food	fairly straight; slow
hMAPT (Tau) Wildtype	High-sugar Diet	straight; fast
hMAPT-4Q	Normal food	zig-zag; slow
hMAPT-4Q	High-sugar Diet	fairly straight; fast
hMAPT-4R	Normal food	zig-zag; slow
hMAPT-4R	High-sugar Diet	fairly straight; fast

Table 2. Qualitative analysis of climbing patterns in female *Drosophila*

Female Fly Strains	Treatment	Average climbing pattern
Oregon-R Wildtype	Normal food	straight; slow
Oregon-R Wildtype	High-sugar Diet	zig-zag; fast
GMR-hTau	Normal food	straight; slow
GMR-hTau	High-sugar Diet	fairly straight; fast
GMR-hTau-hGSK3β	Normal food	fairly straight; slow
GMR-hTau-hGSK3β	High-sugar Diet	fairly straight; fast
hMAPT (Tau) Wildtype	Normal food	fairly straight; slow

hMAPT (Tau) Wildtype	High-sugar Diet	straight; fast
hMAPT-4Q	Normal food	fairly straight; slow
hMAPT-4Q	High-sugar Diet	straight; fast
hMAPT-4R	Normal food	zig-zag; slow
hMAPT-4R	High-sugar Diet	straight; fast

Table 3. Relationship between different male fly strains and climbing distance

Male Fly Strains	Treatment	Number of flies in the vial	Average number of flies that passed 7 cm (3 Trials)
Oregon-R Wildtype	Normal food	30	15.00
Oregon-R Wildtype	High-sugar Diet	30	18.33
GMR-hTau	Normal food	30	9.56
GMR-hTau	High-sugar Diet	30	10.33
GMR-hTau-hGSK3β	Normal food	30	6.00
GMR-hTau-hGSK3β	High-sugar Diet	30	17.33
hMAPT (Tau) Wildtype	Normal food	30	10.33
hMAPT (Tau) Wildtype	High-sugar Diet	30	15.00
hMAPT-4Q	Normal food	30	5.67
hMAPT-4Q	High-sugar Diet	30	18.33
hMAPT-4R	Normal food	30	8.00
hMAPT-4R	High-sugar Diet	30	11.56

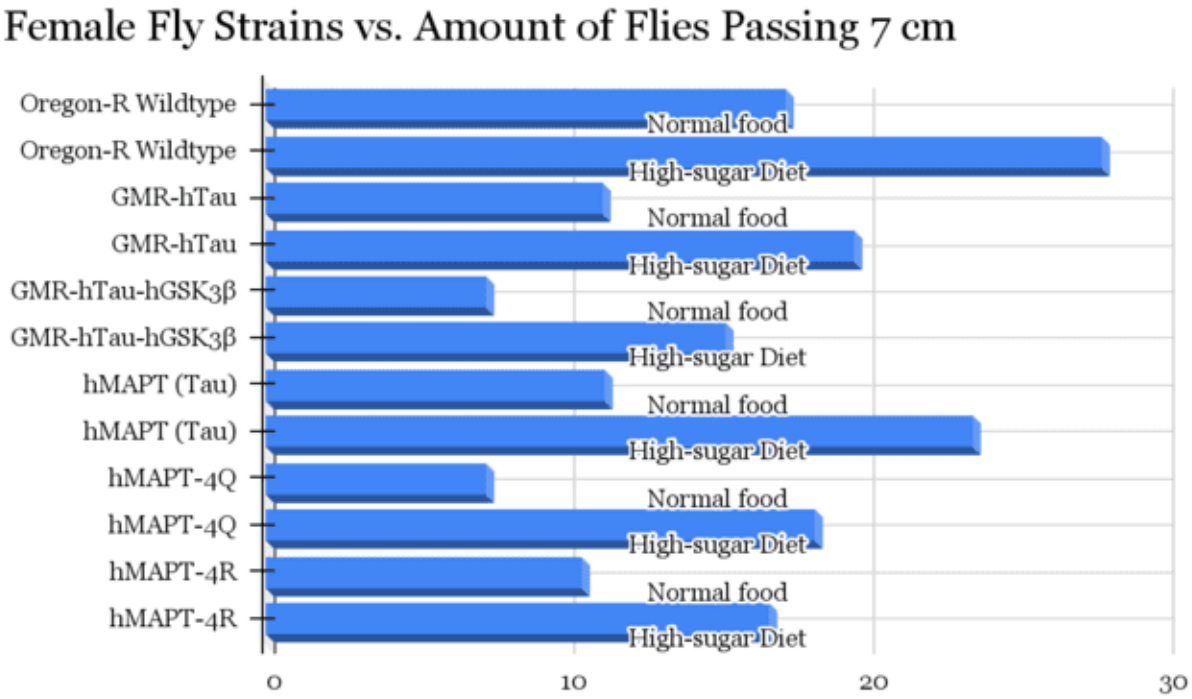
Table 4. Relationship between different female fly strains and climbing distance

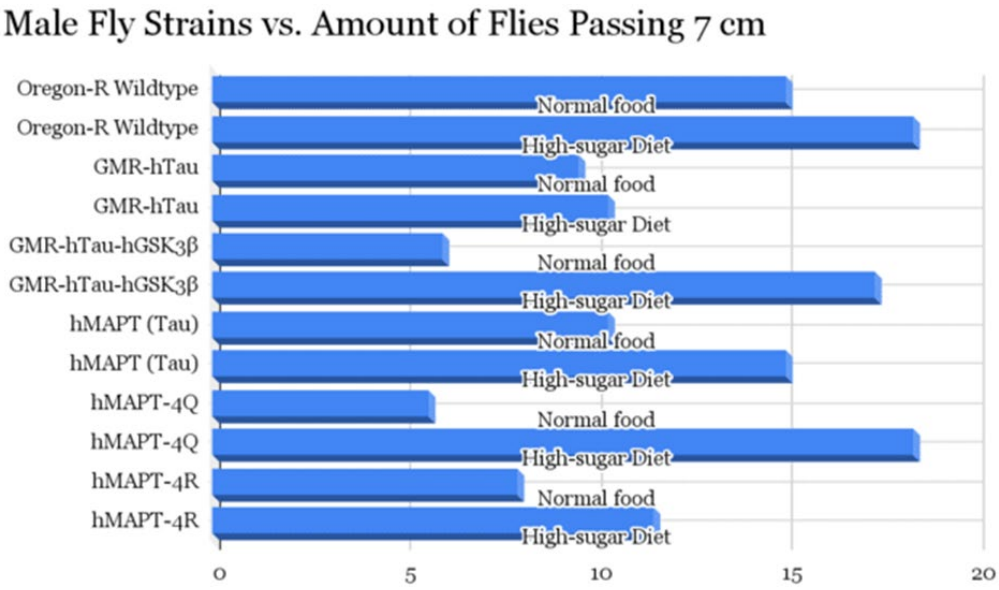
Female Fly Strains	Treatment	Number of flies in the vial	Average number of flies that passed 7 cm (3 Trials)

Oregon-R Wildtype	Normal food	30	17.33
Oregon-R Wildtype	High-sugar Diet	30	27.87
GMR-hTau	Normal food	30	11.23
GMR-hTau	High-sugar Diet	30	19.66
GMR-hTau-hGSK3β	Normal food	30	7.33
GMR-hTau-hGSK3β	High-sugar Diet	30	15.33
hMAPT (Tau) Wildtype	Normal food	30	11.33
hMAPT (Tau) Wildtype	High-sugar Diet	30	23.56
hMAPT-4Q	Normal food	30	7.33
hMAPT-4Q	High-sugar Diet	30	18.33
hMAPT-4R	Normal food	30	10.56
hMAPT-4R	High-sugar Diet	30	16.76

Table 5. Relationship between male and female eye development

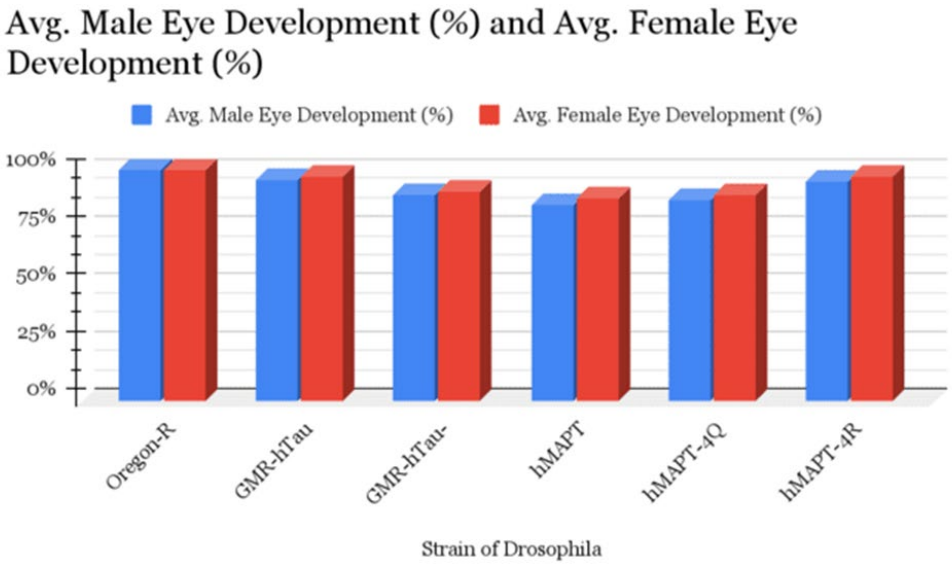
Strain of *Drosophila*	Oregon-R Wildtype (Control)	GMR-hTau	GMR-hTau-hGSK3β	hMAPT (Tau) Wildtype	hMAPT-4Q	hMAPT-4R
Avg. Male Eye Development (%)	100 	96 	89 	85 	87 	95 
Avg. Female Eye Development (%)	100 	98 	91 	88 	89 	97 





Graph 1 & 2. Male vs. female flies passing 7 cm in 7 seconds. In the bar graphs above, the male flies weren't able to pass the 7 cm boundary as fast as the female flies. The x-axis represents the amount of flies able to pass the 7 cm boundary, while the y-axis shows the different *Drosophila* strains. When fed a high-sugar diet, in all the strains, 15.15 males were able to climb past 7 cm, while 20.25 females climbed the same distance. The highest number of male flies that were able to climb past 7 cm were 18.33 flies, and 27.87 females, which stood out as an outlier. However, the lowest amount of male flies that were able to climb past 7 cm was 11.56 flies with 15.33 females as the lowest amount too. When comparing both male and female flies through a statistical correlation test, I found that the p-value, or probability value, is .001757, which is less than the accepted significance value of .05. I used a correlation test to be

able to correlate the results of my experiment (climbing and eye development) to male and female Alzheimer's resistance. This was the best test to use because it is the only test that explains a casual relationship. As seen in the graphs above, the range between the male flies and the female flies differs by 10 flies, making the female flies more consistent in being able to climb past 7 cm in 7 seconds.



Graph 3. Relationship between male eye development vs. female eye development. In the bar graph above, the male flies 'eye development decreased slightly more than the females 'eye development in the span of a week. The x-axis shows the different strains within males and females, while the y-axis represents the average percentage of eye development in the fruit flies. In the Oregon-R Wildtype control strain, the eyes developed normally. On average, the percentage covered in the eyes, excluding the control, for males was 90.4% while the females was 92.6%. The highest percentage covered for males was 96% and the lowest was 85%. However, the highest percentage covered for females was 98% and the lowest was 88%. According to the graph, the GMR strains had about the same development. The lowest percentage was in the male hMAPT Wildtype eye, which was 85% developed, while the highest percentage (not including the control) was in the female GMR-hTau eye which was 98% developed. While there were slight differences, the p-value was .000067, meaning the results are significant. As stated before, the correlation test was the optimal statistical test for this experiment because of its ability to correlate causal relationships.

Data Analysis

Revisiting Hypothesis

Now that the data is present, the null hypothesis can be revisited and either rejected, supported, or partially supported.

Null Hypothesis: A high-sugar diet has no impact on Alzheimer's severity in *Drosophila*.

Alternate Hypothesis: A high-sugar diet has an impact on Alzheimer's severity in *Drosophila*.

My study successfully rejects the null hypothesis as the p-value was found to be .001757 (climbing assay) and .000067 (eye development). The accepted significance value in this study's field is .05 for both the eye measurement and the climbing patterns, so because the p-value is smaller than the significance value, there is convincing evidence that a high-sugar diet has a positive impact on Alzheimer's in *Drosophila*. As a reminder, the purpose of this experiment was to see whether a high-sugar diet had an impact on the severity of Alzheimer's.

Male Versus Female

After the experiment, my data supported the claim that females were more resistant to progression of Alzheimer's compared to males based on the significance tests and statistical averages. Unusually, Hanamsagar et al. (2016) concluded that women had "less favorable outcomes and suffer from a more precipitous drop in health status compared to men" in humans. My data showed the opposite of these findings. The female flies climbed consistently faster than males, suggesting that females are more resilient in recovering from AD neurodegeneration when fed a high-sugar diet.

Contrary to expectations, other researchers suggested that males were affected by Alzheimer's more than their counterparts. Pike (2016) claims that "men and women are affected by AD differently, both in terms of disease development and progression." Like Pike's claim, my research suggested the same, but it filled in the gap by suggesting that males seemed to be more affected by the females. These observed sex differences may stem from inherent biological variations in hormone levels, genetic predispositions, or metabolic responses due to dietary factors which can also be seen in humans. For example, estrogen in females has been shown to possess neuroprotective properties and may confer resilience against Alzheimer's pathology in females (Brotfain et al., 2016). Moreover, differences in energy metabolism and nutrient utilization between male and female *Drosophila* could influence disease susceptibility, with females potentially exhibiting more efficient metabolic adaptations to high-sugar diets that mitigate neurodegeneration.

My findings are important in considering sex-specific factors on disease mechanisms and targeted therapeutic strategies for Alzheimer's. By clarifying these mechanisms driving sex disparities in disease susceptibility, I identified novel therapeutic targets that are tailored to individual sex differences. Furthermore, the observed sex differences in response to dietary interventions highlight the need for personalized approaches in Alzheimer's prevention and treatment, emphasizing the importance of accounting for sex-specific factors in clinical trials and healthcare practices to optimize outcomes for male and female patients.

Climbing Patterns

Looking at the climbing patterns of female *Drosophila* compared to males raises intriguing questions about the potential underlying factors that drive this sex disparity. Post-experiment, I analyzed the gathered data to identify any discernible differences in climbing performance between the groups. The data showed that the flies fed a high-sugar diet had irregular climbing patterns compared to the baseline flies. Qualitatively, the female *Drosophila* were able to climb up in a straight line, but the males did not. On average, 20.25 out of 30 female *Drosophila* climbed past the boundary in 7 seconds while 15.15 out of 30 males did as well. This analysis fills in the gaps for potential effects of the high-sugar diet on the flies 'mobility and neurological health. Researchers say that excessive sugar intake was significantly associated with AD risk in women, which contradicts my data (Liu et al., 2022). The climbing assay served as a key metric to evaluate the physiological effects in males and females, shedding light on dietary influences on locomotive function.

One possible explanation is the influence of sex-specific physiological/behavioral differences on locomotor abilities. Females may possess inherent biological characteristics or genetic factors that confer superior climbing performance, such as muscle strength or agility. Additionally, variations in hormone levels may impact motor function and neuromuscular coordination, contributing to the observed differences in climbing behavior between male and female flies. Furthermore, environmental factors within flies may play a role in sex-specific behaviors, highlighting the complexities within genetic and environmental influence on development in *Drosophila*.

Eye Development

Eye development in the flies fed high-sugar was not as impacted as their climbing patterns. I compared all the different fly strains to the normal wildtype. My data showed that the females had slightly more development in their eyes compared to the males. There wasn't a large difference, however the average development for males was 92% while the females was 93.8%. This percentage suggests that females had slightly higher eye tissue development rate when fed a high-sugar diet compared to males also fed the same.

The observed sex-specific differences in eye development may reflect underlying variations in metabolic responses to the high-sugar diet, hormonal influences, or genetic predispositions. Interestingly, the tau protein, implicated in AD pathology, is expressed in the eyes of *Drosophila* and plays a role in regulating microtubule stability and neuronal function. Therefore, examining the expression levels and distribution patterns of tau protein in the eyes of female and male *Drosophila* provided valuable insights into the link between metabolic perturbations, eye development, and AD pathology. This analysis involved visualizing tau protein localization and quantifying protein expression levels in the eye tissues of *Drosophila* subjected to different dietary conditions. My data showed a difference in eye development. Understanding how diet-induced metabolic changes influence tau protein expression and eye development in a sex-specific manner could offer novel insights into the mechanisms underlying AD and inform potential therapeutic strategies targeting metabolic pathways.

Limitations

Human error is an inherent limitation within experimental procedures. Due to the qualitative visual analysis, there could have been discrepancies between the actual and the analyzed patterns of climbing. Because I subjectively analyzed the flies' climbing patterns, the pattern was not objective. This could affect my data and create discrepancies in the flies 'climbing patterns, therefore decreasing accuracy and suggesting that the flies worsened when fed a high-sugar diet. However, my quantitative analysis of the climbing patterns counters the subjectivity, creating highly reproducible results.

Specific limitations would be that the strains cannot account for *all* strains of *Drosophila*. The strains have different genetic modifications, so their results cannot translate to all organisms. However, because of the hTau gene, I can translate my findings to human brain functions. The genetic differences between each of the strains cannot account for all human genomes, limiting the data's generalizability. Genetic factors in different populations can further contribute to variability among strains, thus, caution must be exercised when extrapolating findings to draw conclusions about broader fruit fly populations, emphasizing the need for diverse genetic backgrounds to better understand the complexities in neuro-biology of *Drosophila*.

Furthermore, when doing the climbing assay, some of the flies did not fall directly to the bottom. This gave them an advantage in climbing to the top of the vial which could have limited our accuracy in counting the number of flies that passed the boundary. Flies that inadvertently gained advantages in climbing due to their initial positioning may artificially inflate measures of climbing ability. This can lead to biased conclusions and inaccurate interpretations of the data, potentially obscuring genuine differences in climbing performance among experimental groups. Moreover, the inconsistency introduced by varying starting positions complicates comparisons across studies and undermines the study's reliability and reproducibility. Therefore, I had to diligently address this limitation to minimize its influence on my data and ensure the validity of my results.

Implications

Assessing the severity of AD carries significant implications for understanding the disease and exploring potential therapeutic interventions. AD is characterized by the accumulation of abnormal protein aggregates, such as tau tangles,

leading to cognitive decline and neuronal dysfunction. High-sugar diets have been linked to various metabolic disturbances, including insulin resistance and oxidative stress, which are also implicated in AD. By subjecting *Drosophila* fed a high-sugar diet to climbing assay and eye development analysis, I am able to investigate how dietary factors influence disease progression and behavioral outcomes, providing valuable insights to complex interplay between metabolism, neurodegeneration, and cognitive function. In my data, this correlation is supported, meaning that a high-sugar diet does decrease the severity of Alzheimer's.

Along with investigating the influential factors, utilizing flies in climbing assays to investigate the effects of a high-sugar diet on AD can provide crucial insights into potential preventative measures. I can identify lifestyle interventions and dietary modifications that may mitigate the risk of Alzheimer's progression. This research could inform public health strategies aimed at promoting healthy dietary habits and reducing the burden of AD on individuals and healthcare systems. Additionally, understanding the links between diet and neurodegeneration in a simple model organism, like *Drosophila*, can inspire innovative approaches for personalized medicine and targeted interventions tailored to individual genetic profiles, ultimately paving the way for more effective strategies to combat AD.

Moreover, the use of *Drosophila* as a model organism offers several advantages for studying AD and evaluating potential therapeutic interventions. *Drosophila* share many fundamental biological processes and genetic pathways with humans, making them a valuable tool for investigating disease mechanisms and screening candidate drugs/diets in a cost-effective and high-throughput manner (Kasanin et al., 2022). By manipulating the fruit fly genome and administering dietary supplements, I am able to target specific molecular pathways involved in Alzheimer's pathology and assess their impact on climbing behavior and neuronal function. This approach enables the rapid identification of novel dietary targets and the evaluation of potential treatments for AD, accelerating the proper diet discovery process and facilitating the translation of preclinical findings into clinical applications.

Areas for Future Research

Future research exploring high-sugar diets on the severity of AD in *Drosophila* expand into several directions. Firstly, investigating these effects in more complex organisms, such as mice or non-human primates, could provide valuable insights into improving fruit fly models. Examining how diets, including variations in sugar content and composition, impact disease progression and phenotypic outcomes could offer a comprehensive understanding of the role dietary factors in Alzheimer's. Furthermore, exploring the effects of high-sugar diets on the aggregation of different Alzheimer's protein species, such as alpha-synuclein or TDP-43, could shed light on the broader molecular mechanisms underlying neurodegeneration and cognitive decline. By integrating approaches from diverse disciplines, including genetics, neuroscience, nutrition, and aging research, future studies have the potential to uncover novel therapeutic targets and strategies for mitigating AD progression and improving outcomes for those affected.

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