Spring 2024

Effect of Downregulating Lactate Dehydrogenase in Alzheimer's Disease using a Drosophila Model

Hannah Higgins
Coastal Carolina University, hlhiggins@coastal.edu

Effect of Downregulating Lactate Dehydrogenase in Alzheimer's Disease using a Drosophila Model

By

Hannah Higgins

Biochemistry

Submitted in Partial Fulfillment of the
Requirements for the Degree of Bachelor of Science
In the HTC Honors College at
Coastal Carolina University

Spring 2024

Louis E. Keiner
Director of Honors
HTC Honors College

Fang Ju Lin, PhD
Associate Professor
Biology
Gupta College of Science

Abstract

According to the CDC, approximately 5.8 million Americans suffered from Alzheimer's disease (AD) in 2020. AD is characterized by progressive memory loss and typically develops after the age of 60. There are many studies that suggest different causes of Alzheimer's such as oxidative damage, amyloid plaque buildup, and metabolic dysfunction. Lactate Dehydrogenase (LDH) is an enzyme that is found in many tissues within the body and functions within cellular metabolism. The enzyme catalyzes the reversible reaction that converts lactate to pyruvate coupled with the reduction of NAD+ to NADH. Pyruvate is then used in the Krebs cycle to create ATP. In a study conducted by the University of Western Ontario, it was observed that Alzheimer's patients showed an increase in LDH gene expression and enzymatic activity. With this knowledge, the contents of this research use fruit flies implanted with human Alzheimer's (Drosophila melanogaster) to observe the effects of LDH RNAi. To further model the disease, the flies were crossed with a strain that carried human Aβ42. To measure the effects of the LDH RNAi the lifespan of the flies and their locomotor function was monitored. It was shown that downregulating RNAi through LDH inhibitors increased the life span of the flies as well as their locomotor function.

Introduction

Scientific advancements have changed the way of life of developed countries in such ways that they would be unrecognizable to people from just a hundred years ago. The most important types of advancements have been those made in the medical field. From medications to procedures, changes in medicine have allowed people to live healthier and longer lives. For instance, in the early nineteen hundreds, almost no country had a life expectancy that was higher than 50 years of age (1). As of 2010, life expectancy has increased to approximately 80 years of age, with U.S.

males surviving to 78 years and U.S. females to 83 years (1). Such an increase was greatly aided by dwindling mortality rates of heart disease, specifically as the disease affects those in the older category (1). This means that once people reach a higher age, such as 65 and older, they are even more likely to live longer (1). Life expectancy is even expected to increase in the future as many health issues like strokes and heart disease are on the decline (1). However, with this increase in life expectancy comes the new risk of Alzheimer's disease.

Alzheimer's disease is a neurological disorder that generally appears in a person in their mid to late 60's (2). Although, in rare instances the disease can appear in a person's 30s, if this occurs it is known as early-onset Alzheimer's (2). The disease can present differently in each individual, but the first symptoms are generally memory problems (2). The progression of the disease is perceived in four stages: preclinical, mild, moderate, and severe (2). The preclinical stage, which is associated with the buildup of amyloid plaques within the brain, is thought to start almost a decade before symptoms appear (2). By the time symptoms appear, the individual has entered the mild stage. In this stage the individual can appear healthy, but they may have memory loss, poor judgment, trouble remembering dates or locations, an increase in misplacing objects, personality changes, as well as other symptoms (2). The presence of these symptoms is often why a person is evaluated and diagnosed with Alzheimer's. From there, the symptoms worsen as the individual enters the moderate stage. This stage is where the individual can forget their history, they have trouble speaking and understanding language, they often have problems remembering family or friends, and much more (2). Finally, the person will enter the severe stage, which is generally characterized by more physical symptoms. In this stage the person may have seizures, trouble swallowing, incontinence in both bladder and bowel, as well as the inability to speak (2).

It is estimated that 6.7 million Americans over the age of 65 are currently suffering from Alzheimer's disease (3). It is also predicted that this number will rise to 13.8 million in the year 2060 (3). Additionally, from 2000 to 2019 deaths from Alzheimer's disease increased by 145% while other diseases like heart disease, stroke, and HIV decreased (3). In 2019 Alzheimer's was listed as the sixth leading cause of death within the United States, however, in 2020 it dropped to seventh, as COVID 19 was now in the ranks (3). Those afflicted with the disease require a great amount of care. This care can be as simple as observing the individual for their safety or it can be as extreme as performing all acts of daily living for them. In just 2022, approximately 11 million people, including family members and any other unpaid help, provided approximately 18 billion hours of care (3). The value of this unpaid work has been valued at roughly $339.5 billion (3). The emotional toll that the disease can take on families is also incredibly difficult. Because the disease is often marked by personality changes, someone who once was calm and loving may now be angry and violent. As the individual loses more of their memory, they may not recognize friends or family, and become upset when corrected. In addition to this the cost of care for an individual with Alzheimer's is incredibly high. In 2023 alone the total payments made for Alzheimer's related care was $345 billion (3). There is also concern about the number of health care providers available to provide treatment (3). This concern was further amplified by the decrease in providers after COVID 19. It is reasons like this, as well as the high prevalence and mortality that has led to countless studies on the disease. Although these studies have not led to a cure for Alzheimer's, they have provided insight into how the disease develops. The most common theories include the development of amyloid plagues, the occurrence of oxidative stress, and/or the presence of metabolic dysfunction.

As stated previously, in the preclinical stage an individual's brain composition changes as amyloid β plaques build up. Amyloid beta is a product of the breakdown of the membrane protein known as amyloid beta-precursor protein (APP) (4). The cleavage of APP is carried out by beta-amyloid cleaving enzyme (BACE) and gamma-secretase (4). This results in two products, one that is usually 40 amino acids long and another that is usually 42 amino acids long (4). These are known as amyloid beta 40 and amyloid beta 42 (4). The former is more commonly made by the neurons than the latter (4). While amyloid beta 40 is more prevalent, amyloid beta 42 has two C terminal residues that are hydrophobic, leading them to form amyloids (4). Amyloids are formed by the misfolding of proteins that create a specific cross-β structure (4). This initiates the misfolding of other proteins and forms filiform polymers with the other β sheets of the misfolded proteins (4). There are three kinds of amyloid β plaques seen in Alzheimer's patients including diffuse, focal, and vascular deposits (5). Diffuse deposits are loosely compressed filaments that can be found in many areas of the brain such as the striatum, brainstem, entorhinal cortex, and cerebellum (5). In contrast focal deposits are densely packed and have a circular core of dense filaments surrounded by loose filaments (5). These plaques are mainly found in the hippocampus and the cerebral cortex (5). Vascular deposits are found within the blood vessels of the brain (5). It has been found that the three kinds of plaques can arise from different amyloid β plaques. The diffuse deposits and the loose filaments of focal plaques are mainly formed by amyloid β 42 (Aβ42) whereas the core of focal plaques and vascular plaques are formed by amyloid β 40 (Aβ40) (5).

It has been recorded that the filaments of the plaques can be grouped based on a difference in structure. The first group of filaments is denoted as Type I and are formed by two S shaped protofilaments that intertwine with each other (5). The S shaped portion wraps around

two hydrophobic clusters, which are formed by the N-terminal and C-terminal parts of several side chains (5). The second group is denoted as Type II. In this type, the core of the filament is made of four beta strands (5). Other residues on these filaments have the same S shape seen in Type I (5). Type II filaments are also smaller than Type I filaments (5).

Aβ's direct role in the progression of Alzheimer's is not yet known but many experiments have pointed to toxicity of Aβ's. It is suggested that Aβ's can cause synaptic damage based on their ability to interact with receptors found at synaptic terminals, though it is not known if the damage occurs by a pre or postsynaptic mechanism (6). Due to Aβ's ability to bind to a wide range of cell receptors, Aβ's can cause the initiation of damaging signaling pathways (6). Amyloid betas have also been found intracellularly in places like the Golgi apparatus, lysosomes, and the mitochondria (6). In terms of the mitochondria, APP is not found within the membrane, making it particularly unusual that Aβ's are present (6). There are theories that could be entering the organelle by binding to transport proteins (6). Others think that calcium could be interacting with Aβ's, causing the formation of membrane pores (6). Either way, it has been shown that the presence of Aβ's can stop the synthesis of mitochondrial ATP (6).

Furthermore, Aβ's has been shown to bind to all cellular membranes and their binding has led to an increase in intracellular calcium (6). Such an increase is related to cell damage and death (6). Aβ's has also been shown to have an oxidative effect. The molecule has metal binding sites and thus the ability to reduce copper (II) and iron (III) to copper (I) and iron (II) (6). Oxygen will then react with the reduced metals to create a superoxide anion, which then interacts with hydrogen to create hydrogen peroxide (6). The creation of hydrogen peroxide can lead to cell damage at high concentrations (7).

Another theory about the progression of Alzheimer's is the accumulation of oxidative stress. Oxidative stress is the imbalance between the concentration of reactive oxygen species (ROS), also known as free radicals, and antioxidant mechanisms (8). Reactive oxygen species occur naturally in cellular metabolism, specifically from mitochondrial respiration (9). During the process of mitochondrial respiration electrons are transferred by the electron transport chain and in transfer some electrons may be lost (9). There are also other enzymes that create ROS's. Due to ROS's being able to naturally occur, there are also detoxification systems that keep the ROS concentration low (9). An example of a detoxification system is superoxide dismutase, an antioxidant enzyme. This enzyme works by transferring electrons from the highly toxic superoxide anion to the metal active sites of the enzyme (9). Catalase is another enzyme that has a function as an antioxidant. This enzyme's active sites are heme groups that take the electrons form hydrogen peroxide, creating the harmless by product of water (9). Antioxidants are not always enzymes, for example, vitamin c can function as an antioxidant (9). If the concentration of ROS is allowed to get too high, the balance between ROS and antioxidants will be thrown off and damaging effects can occur.

ROS have an unpaired electron that makes them unstable therefore, to stabilize themselves the molecules will take an electron from other biomolecules such as DNA, proteins, and lipids (9). In DNA, the loss of an electron when exposed to a ROS, leads to breaks in the DNA (9). DNA is broken because a reactive oxygen species can interact with guanine, creating 8-oxoguanine which can in turn pair with cytosine as well as adenine (9). For proteins, exposure to ROS can lead to loss of function as the structure of the protein is modified (9). Finally, as for lipids, ROS interaction leads to lipid peroxidation which then leads to cell membrane damage (9). All these effects can be detrimental to cells. However, there are systems that work to fix

damage caused by oxidation. Based on these facts, a theory known as “The Free Radical Theory of Aging” was created. The theory proposes that the effects of aging are due to the damage caused by reactive oxygen species (9). As previously mentioned, Aβ’s can play a role in oxidative stress as it leads to the creation of hydrogen peroxide, a reactive oxygen species.

Finally, metabolic dysfunction is also attributed to the development of Alzheimer’s. One area of metabolic dysfunction relates to diabetes. After a review of Alzheimer’s patients, it was found that 80% of patients also had type two diabetes or some form of unnatural fasting glucose results (10). Within Alzheimer’s individuals, it was found that they have a larger number of insulin receptors in the brain than other individuals (10). This could be an indication of the decreased response to insulin, which is also seen in people with diabetes (10). Additionally, hyperinsulinemia causes a decrease in insulin degrading enzyme (IDE), an enzyme that helps in the degradation of Aβ’s (10). Ghrelin, a hormone that causes the feeling of hunger, is also under examination for its role in Alzheimer’s (10). There are preliminary studies that have shown the injection of ghrelin into rats led to an increase in memory, alluding to the hormone’s possible role in mental function (10). With this knowledge Alzheimer’s patients were evaluated for their ghrelin levels and some were found to have altered levels of the hormone (10). Some Alzheimer’s patients also have higher levels of adiponectin within their plasma and cerebrospinal fluid (10). Adiponectin is another hormone that works in the regulation of insulin, although little research has been done on its correlation with the disease (10).

An enzyme known as lactate dehydrogenase (LDH), has also been associated with Alzheimer’s patients. The enzyme catalyzes the reversible reaction of pyruvate to lactate, this reaction is coupled with NADH (11). Pyruvate is the final product of glycolysis that will then enter oxidative phosphorylation if oxygen is present (11). If oxygen is not present, pyruvate will

be converted into lactate, which is where the energetic breakdown stops (11). Lactate will then need to be transported to the liver for LDH to convert it back into pyruvate (11). As a catalysis, LDH makes the conversion of pyruvate to lactate occur 14 times faster than the reaction would occur without it (11). To perform this reaction NADH first binds to the enzyme which will then cause the binding of lactate to the enzyme (11). Once that is complete, a hydride ion from NADH is transferred to pyruvate at the C2 carbon to create lactate (11). The enzyme is regulated in three ways, allosterically, on the substrate level, and by transcriptional regulation (11).

While LDH is formally only thought to play a role in energetic metabolism, high levels of the enzyme have been observed in both aging and Alzheimer's diseases (12). This led researchers to question the enzymes function in the progression of the disease. A research group led by Dani Long investigated the expression of LDH in Drosophila melanogaster, commonly known as the fruit fly, to answer this question. The group noted that flies that overexpressed the LDH enzyme had a lifespan that was roughly 17 days shorter than the control flies (12). It was theorized that this shorter lifespan could be related to brain neurodegeneration caused by LDH (12). To see if this was accurate, the creation of vacuoles within the brain was monitored as they serve as a measurement of neurodegeneration in neurological disease, like Alzheimer's (12). It was observed that flies with an increased amount of LDH had an average number of vacuoles that was much higher than in the control flies (12). With these results, LDH was associated with degenerative aging and to be a factor in the life span of flies (12).

High levels of LDH may be a problem because of the newfound importance of lactate. Originally, it has been thought that glucose was the primary source of energy used within the brain (13). In the early 1990s studies were conducted that have contested this original claim, proposing that lactate is the central fuel source for neurons (13). This theory came to be based on

the study of astrocytes, glial cells that work in the brain to regulate synaptic ion and neurotransmitter levels as well as defend against oxidative stress (13). Astrocytes shape also allow them to provide energetically favorable substrates from the capillaries to neurons (13). Most importantly however, astrocytes have the ability to store glycogen, which is the brain's source of energy during periods of low glucose levels (13). When energy is required, glycogen will be broken down to lactate by glycogenolysis (13). These cells also use the GLUT1 transporter to uptake glucose which they will in turn convert to lactate (13). This will be released into the extracellular space to then be captured by neurons and changed to pyruvate by LDH (13). The pyruvate will then enter the citric acid cycle and the following molecules will then fuel oxidative phosphorylation. Finally, astrocytes can also take up glutamate which will cause the cells to use even more glucose (13). All the functions have led researchers to create the astrocyte neuron lactate shuttle (ANLS) model, which sees a large synthesis of lactate that will then be used by neurons for oxidative and nonoxidative ATP synthesis (13).

Furthermore, there are studies that suggest lactate is influential in the creation of long-term memories. These studies began with the knowledge that the creation of long-term memories is an energetically demanding process as it requires the activation of genes, protein synthesis, and the formation of synaptic connections (13). Through studies that inhibited glycogen phosphorylation it was observed that long term memory was also inhibited, indicating that glycogenolysis is crucial in the formation of long-term memories (13).

The work conducted by Long specifically investigated the overexpression of lactate dehydrogenase and its relationship to neurodegeneration. Since neurodegeneration is associated with Alzheimer's, the research of this paper focused on the downregulation of LDH in an Alzheimer's model. To accomplish this, LDH RNAi was used to decrease the expression of

LDH. RNAi stands for double stranded RNA mediated interference, and it is a process that silences the expression of genes (14). Silencing occurs either by transcriptional suppression, often referred to as transcriptional gene silencing (TGS), or by initiating a breakdown of RNA, referred to as post-transcriptional gene silencing (PTGS) or RNA interference (RNAi) (14). PTGS/RNAi was first seen in plants but has since been observed in organisms from protozoa to human cells (14).

Several different terms were used to describe this RNA breakdown based on the organism that was studied. In plants RNA silencing was described as PTGS, in animals it is described as RNAi, and in fungi it was termed quelling (14). However, all of these are a part of gene regulation and carry out their function with similar mechanisms. First, RNA silencing requires an initiator which comes in the form of double stranded RNA (dsRNA) (14). Then the target RNA is broken down via a homology-dependent system (14). This system is defined by the creation of small RNA (siRNA), which once made will come together to become dsRNA (14). Further studies showed that several proteins and crucial enzymes are found when RNA silencing occurs. One of these molecules was identified to be a part of the RNase III family, a nuclease that recognizes dsRNAs and cuts the sequence to then yield a 3’ overhang and a 5’-phosphate and 3’-hydroxyl termini (14). Based on the enzymes ability to cut dsRNA into perfectly unique siRNA it was termed Dicer (DCR) (14). The Dicer enzyme consists of four domains. These include the amino-terminal helicase domain, the dsRNA binding domain, a RNase III motif, and a domain known as PAZ due to its similarity to other proteins like Piwi and Zwille (14). The mechanism by which Dicer cleaves is theorized to be done by the RNase III domain (14).

The model chosen for this research was the Drosophila melanogaster. This model organism is commonly known as the fruit fly and is used widely throughout research. Fruit flies had their rise to fame because of research conducted by Thomas Hunt Morgan which used the fly's white gene on the X chromosome to prove the theory that chromosomes are responsible for inheritance (15). From there the genes of the fruit fly were found to be homologous to those within humans (15). Based on the genetic similarities as well the short lifespan and ability to create large sample sizes, the fruit fly became an excellent model organism. Furthermore, the development of molecular techniques such as CRISPR/Cas9 have made it possible to create various mutations within organisms. With this ability, lines of fruit flies can be created to represent numerous disease models.

Development of CRISPR/Cas9 began with the discovery of clustered regularly interspaced short palindromic repeats (CRISPR) within *E. Coli* (16). These repeats were determined to be a part of the bacteria immune system that allowed the organism to survive infections from virus. This system has been studied and harnessed for gene editing. In terms of gene editing CRISPR/Cas9 has stood out because of its efficiency. The methodology of the system involves a guide RNA (gRNA) as well as a Cas9 protein (17). To begin, the designed guide RNA will recognize the gene of interest, then the Cas9 protein will create a double stranded break in the DNA (17). It is important to note that Cas9 will only cut if a short DNA sequence known as PAM is upstream of the target sequence (17). After the break occurs, it will be fixed by either non-homologous end joining, or homology directed repair. Non-homologous end joining works by joining the ends of the cut DNA (17). This piecing together can lead to DNA mutations such as insertions or deletions (17). For gene editing, the second mechanism is the desired outcome as it uses a complementary strand of DNA to fix the break. Knowing this, a

template DNA of the desired genetic change can be made to be used for homology directed repair (17). This form of gene editing is commonly used to create mutations in model organisms such as the fruit fly.

Other research has been done in relation to LDH with a drosophila Alzheimer's model. Research led by Teresa Niccoli investigated how the activation of transcription factor 4-dependent LDH could serve as a protection against amyloid beta toxicity. To conduct this research the fly line *UAS-ArcAß42/+; elavGS/+* was created to simulate Alzheimer's disease (18). As a way of determining responses to amyloid beta plaques transcriptional responses were monitored at several different ages (18). It was noticed that many of the transcriptional changes in the presence of amyloid beta 42 seen in older flies were similar to the responses seen in younger flies (18). From this, it was theorized that this was due to general brain responses to early disease pathology (18). The researchers also compared microarray assays of the fly brains to those of Alzheimer's patients. It was found that many genes were expressed differently while still being regulated (18). Most importantly, it was observed that LDH was conserved and then incorrectly expressed when amyloid beta plaques occurred after the exposure to activating transcription fact 4 (ATF4) (18). Through this research LDH was identified as a key transcriptional responder to amyloid beta 42 toxicity for young and old flies (18). Interestingly, more LDH was synthesized in younger flies than older flies, but it could not be determined if LDH served as a way to protect against the damage from amyloid beta 42 (18). The results of the study show that amyloid beta 42 leads to the overexpression of LDH in flies with ATF4 (18). For a better understanding of LDH's role, ATF4 RNAi was used to stop the synthesis of LDH (18). Doing this led to the death of the flies, indicating that LDH possess a neuroprotective effect when amyloid beta 42 is present (18).

In addition to this, research has been conducted to observe the levels of cerebral lactate and the relevance they have to neurological damage. This research was led by Mao Zhang, and an Alzheimer's mouse model was used (19). More specifically, the model contained the amyloid precursor protein and presenilin1 (19). It is important to understand that lactate transport is possible because of three monocarboxylate transporters denoted as MCT1, MCT2, and MCT4 (19). MCT1functions generally in the release of lactate from myelin, MCT2 functions by allowing lactate to enter into neurons, and MCT4 functions to release lactate from astrocytes (19). As previously mentioned, there is evidence to support that lactate is essential in the formation of long-term memory, therefore these transporters were thought to be crucial in that function. This theory was supported by another study that showed MCT2 levels as well as lactate levels were lower in the cerebral cortex of an Alzheimer's rate model (19). Based on this information, the research of Mao Zhang investigated the potential association of neuronal survival and the changes in lactate metabolism of an Alzheimer's mouse model (19). It was found that lower lactate concentration and lower concentration of the monocarboxylate transporters could cause a stop of lactate transportation from the astrocytes to the neurons (19).

Suppression of lactate dehydrogenase has also been studied. A report led by Anne Le describes how lowering LDH may induce oxidative stress, which as previously said could be implemented in Alzheimer's disease. When LDH was suppressed by short interfering RNA the cells took up more oxygen, suggesting that pyruvate was being pushed through into oxidative phosphorylation (20). An increase in oxygen up take leads to the increase of reactive oxygen species (20). Even though cells normally create ROS, this increased concentration led to cell death (20). After observing these effects with, short interfering RNA was exchanged for FX11 (20). FX11 is a known inhibitor of LDH. In the experiments with FX11, the cells also showed

increase oxygen up take and subsequent death (20). The focus of the described research concerns tumor cells as they are shown to have a high concentration of LDH. This points to an imbalance in LDH being involved in other diseases.

With these studies in mind, it appears as though an increase in lactate dehydrogenase could be a positive occurrence for the protection against Alzheimer's but also has the potential to be harmful, as shown in cancer cells. Using this information, the aim of the research detailed in this paper was to observe the effects of downregulating LDH within an Alzheimer's drosophila model. To accomplish this a fly model with an Alzheimer's phenotype was developed by crossing a strain of flies with human amyloid beta 42 with a control line. Another strain with the Alzheimer's phenotypes was created to also have LDH RNAi. Preliminary data was acquired in terms of the lifespan and locomotor function of the fly lines. The analysis of locomotor function was crucial because the later stages of Alzheimer's see patients lose motor function. Initially, it was expected that the downregulation of LDH would lead to shorter life span and a decrease in locomotor function, aligning with the previously mentioned studies.

Methods and Materials

To determine the effects of downregulating LDH in Alzheimer's, genetically modified Drosophila melanogaster lines were purchased from Bloomington Drosophila Stock Center. These lines include: elav-Gal4 (BL#458), UAS-Abeta42arc mutant (BL#33773), UAS-LDH RNAi (BL#33640), UASmCherry control (BL#35787), and UAS-nGFP control (BL#4776). UAS-RNAi control lines (#60100) were purchased from Vienna Drosophila Resource Center. The first line, elav-Gal4 expresses Gal4. The second line, #33773, expresses human amyloid beta 42. The third line #35787 was crossed with the second line, #33773, to create the Alzheimer's disease representative control group (33773-35787). This line was also crossed with #33640 to

create the Alzheimer's disease with LDH RNAi representative experimental group (33773-33640). The 3587 and 3360 lines were also each crossed with the elav line to serve as further controls, denoted as elav-35787 and elav-33640 respectively. All lines of the flies were kept in either bottles or vials that had Jazz Mix food that was purchased from Thermo Fisher Scientific.

After the flies were bred two points of data were obtained, a lifespan analysis and a negative geotaxis, also known as a climbing assay. For the lifespan analysis five vials, each containing 10 flies, were observed every other day. The number of living flies was documented over a 140-day period. Furthermore, every 5 days the flies were transferred to new vials containing fresh food mix. This analysis was carried out for the created lines; the controls elav-35787, elav-33640, 33773-35787, and the experimental 33773-33640.

The climbing assays were done on newly bred male flies. Groups of 5 flies were placed into vials and ten vials were used for each group for a total of 50 flies per group. This analysis was done once a week on the same day and at the same time. To begin, the flies were transferred to 15mL conical tubes. After allowing the tubes to be undisturbed for one minute, the tube was tapped gently on a block of foam to bring the flies to the bottom of the tube. After this, the tube was gently placed down, and a timer set for 10 seconds. Within those 10 seconds the flies were observed to determine if they could pass the 8cm mark in the time frame, if they could it was recorded as a success. This was reported 5 times per vial, with the flies resting for a minute in between each trial. Climbing assays were conducted for 6 weeks. Another fly line, Oregon Red was included in the climbing assays to survive as a wildtype control.

Results

The results for the lifespan analysis are shown in Figure 1. Each line begins with 100 living flies. The elav-35787 line saw the first deaths at approximately 75 days and a slow decline from there with the final flies dying around 110 days. The elav-33640 saw the first deaths at approximately 65 days with a following steady decline and the final flies almost reaching 120 days. The 33773-35787 line saw the first deaths at approximately 35 days followed by a steep decline and the final flies dying off before 80 days. The 33773-33640 line saw its first deaths at approximately 55 days followed by a steady decline with the final flies dying at approximately 110 days.

The results for the climbing assays are shown in Figure 2. The wild type control, Oregon Red, showed a 90% success rate in week 1 and approximately a 95% success rate in week 2 and week 3. In week 4 the percentage of success dropped slightly, being approximately 85%, before dropping further to 75% in week 5. Finally, week 6 showed a 75% success rate. Line 33773-35787, the Alzheimer's representative group, started week 1 with 95% success rate. In week 2 the percentage dropped slightly to approximately 92% of the flies, reaching the 8cm mark. After that the following weeks saw a steep decline. Week 3 saw a success rate of approximately 75%, week 4 saw approximately 45%, and week 5 dropped to 25%. The final week the percentage of success was less than 5%. Line 33773-33640, the line containing the Alzheimer's phenotype and LDH RNAi, began week 1 with a percentage of success little less than 90%. For week 2 the percentage of success dipped to approximately 85% and week 3 stayed the same. Week 4 the percentage was approximately 87%. For week 5 the percentage of success dropped to approximately 70% and week 6 saw another drop to approximately 55%. It should be noted that during the climbing assays some of the fly lines showed strange movements that can be

described as a circular motion. This observation will be investigated in later experiments using the computer program known as EthoVision.

Discussion

When comparing the experimental fly line 3373/33640, the line with the Alzheimer's phenotype and the LDH RNAi, to the control line 33773/35787, the Alzheimer's phenotype, it was found that the life span of 33640 was significantly longer than that of 35787. With the final flies of the LDH RNAi line dying off at approximately 110 days, their life span roughly matches that of both the elav-35787 and elav-33640. These lines do not contain the amyloid plaques from the 35787 line. The 33773-35787 line had the last flies die at around 80 days, significantly sooner than the experimental group. Such results are indictors that LDH RNAi increases the lifespan of drosophila melanogaster with Alzheimer's.

Furthermore, the locomotor function of 33640 showed little decrease within the six-week period, with week one having a percentage of success of approximately 90% and week six having a success rate of approximately 55%. This is in comparison to 35787 that saw locomotor function decrease steadily over the six-weeks from approximately 95% success to 5% of success. Additionally, when comparing 33640 to the wild type Oregon R, the flies of 33640 are able to reach the 8cm mark almost as well as the controls up until week 5. This data suggests that the downregulation of LDH using RNAi increases the life span and locomotor function of flies.

Based on these two points of analysis LDH RNAi shows a great promise in increasing the lifespan and locomotor function of flies. However, the reason why the downregulation of LDH leads to these results is unclear. These results contrast with other studies that have involved LDH and Alzheimer's disease. Since the findings of this research, more work has been done to analyze

the circular motion observed during the climbing assays. This work involves the use of EthoVison, a computer program that is able to analyze videos to determine things such as peak speed of an organism. The program was used to analyze videos of several fly lines to allow further investigation into the locomotor function of the phenotypes. In the future, the quantification of amyloid protein aggregates will be carried out to determine if the lower concentration of LDH leads to a lower concentration of amyloid protein aggregates. This would be carried out by conducting Western Blot analysis and through immunohistochemistry. Furthermore, the use of an LDH inhibitor, FX11, would be implemented in place of LDH RNAi to see if similar results occur. Finally, there is some interest in investigating how the knockdown of LDH using the inhibitor previously mentioned affects cancer cells.

Conclusion

As the population of the world increases to age, the risk and prevalence of Alzheimer's disease increases as well. Alzheimer's is a severe neurological disease that is often characterized by memory loss. As the disease progresses those who suffer from it will eventually lose the function of their physical abilities. There have been numerous studies on the disease to try and pinpoint the cause of its development and progression. From these studies the most common theory is the aggregation of amyloid beta plaques. However, other theories include oxidative stress and metabolic dysfunction. Metabolic dysfunction was the focus of this research, more specifically, the increase concentration of LDH seen in Alzheimer's patients.

To investigate this, a fly line was created using human amyloid beta 42, recorded as 33773-35787 to mimic the phenotype of Alzheimer's disease. Another line was created that also had the Alzheimer's phenotype but in addition the line had LDH RNAi. This line was recorded

as 33773-33640. While these two lines are the focus of the research, other lines were used as control values, specifically Oregon R as a wild-type control. To observe the effects of LDH RNAi on the flies the lifespan as well as the locomotor function were analyzed. It was shown that the life span of 33640 was significantly longer than that of 35787, with a difference of about 30 days. The results of the climbing assays showed a steep decline in the locomotor function of 35787 as the line had a percentage of success of roughly 95% in week 1 with the percentage dropping to 5% in week 6. Line 33640 on the other hand showed only a slow decline in weeks 5 and 6. The line started with a percentage of 90% success and ended with a percentage of 55% success.

These results indicate that the addition of LDH RNAi leads to an increase in lifespan as well as locomotor function in drosophila melanogaster with Alzheimer's. While the results are incredibly promising, the mechanism as to why lower concentration of LDH increases lifespan and locomotor function is unclear. The results also are in contrast with previous work involving LDH. In the future, amyloid beta plaques would be quantified to determine if lower amounts of LDH lead to fewer amyloid beta aggregates. There is also interest in using an LDH inhibitor known as FX11 in place of LDH RNAi to see if similar results occur. More work has also been done to further study the locomotor function of Alzheimer's phenotypes in flies. This work was conducted using EthoVision, a video tracking computer program that is able to provide data on the exact movements of the organism. The program works by not only tracking the animal within the video, but by analyzing changes on a pixel level as well as in each frame. It is hoped that this study will show a more precise analysis of the changes in the fly lines motor movements.

References

1 Crimmins EM. Lifespan and Healthspan: Past, Present, and Promise. Gerontologist. 2015 Dec;55(6):901-11. doi: 10.1093/geront/gnv130. Epub 2015 Nov 10. PMID: 26561272; PMCID: PMC4861644.

2. "What Are the Signs of Alzheimer's Disease? | National Institute on Aging." *National Institute of Health*, www.nia.nih.gov/health/alzheimers-symptoms-and-diagnosis/what-are-signs-alzheimers-disease. Accessed 28 Apr. 2024.

3. 2023 Alzheimer's disease facts and figures. Alzheimers Dement. 2023 Apr;19(4):1598-1695. doi: 10.1002/alz.13016. Epub 2023 Mar 14. PMID: 36918389.

4. Walker LC. Aβ Plaques. Free Neuropathol. 2020;1:1-31. doi: 10.17879/freeneuropathology-2020-3025. Epub 2020 Oct 30. PMID: 33345256; PMCID: PMC7745791.

5. Yang Y, Arseni D, Zhang W, Huang M, Lövestam S, Schweighauser M, Kotecha A, Murzin AG, Peak-Chew SY, Macdonald J, Lavenir I, Garringer HJ, Gelpi E, Newell KL, Kovacs GG, Vidal R, Ghetti B, Ryskeldi-Falcon B, Scheres SHW, Goedert M. Cryo-EM structures of amyloid-β 42 filaments from human brains. Science. 2022 Jan 14;375(6577):167-172. doi: 10.1126/science.abm7285. Epub 2022 Jan 13. PMID: 35025654; PMCID: PMC7612234.

6. Carrillo-Mora P, Luna R, Colín-Barenque L. Amyloid beta: multiple mechanisms of toxicity and only some protective effects? Oxid Med Cell Longev. 2014;2014:795375. doi: 10.1155/2014/795375. Epub 2014 Feb 5. PMID: 24683437; PMCID: PMC3941171.

7. Heo S, Kim S, Kang D. The Role of Hydrogen Peroxide and Peroxiredoxins throughout the Cell Cycle. Antioxidants (Basel). 2020 Mar 26;9(4):280. doi: 10.3390/antiox9040280. PMID: 32224940; PMCID: PMC7222192.

8. Betteridge DJ. What is oxidative stress? Metabolism. 2000 Feb;49(2 Suppl 1):3-8. doi: 10.1016/s0026-0495(00)80077-3. PMID: 10693912.

9. Shields HJ, Traa A, Van Raamsdonk JM. Beneficial and Detrimental Effects of Reactive Oxygen Species on Lifespan: A Comprehensive Review of Comparative and Experimental Studies. Front Cell Dev Biol. 2021 Feb 11;9:628157. doi: 10.3389/fcell.2021.628157. PMID: 33644065; PMCID: PMC7905231.

10. Cai H, Cong WN, Ji S, Rothman S, Maudsley S, Martin B. Metabolic dysfunction in Alzheimer's disease and related neurodegenerative disorders. Curr Alzheimer Res. 2012 Jan;9(1):5-17. doi: 10.2174/156720512799015064. PMID: 22329649; PMCID: PMC4097094.

11. Farhana A, Lappin SL. Biochemistry, Lactate Dehydrogenase. [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557536/

12. Long DM, Frame AK, Reardon PN, Cumming RC, Hendrix DA, Kretzschmar D, Giebultowicz JM. Lactate dehydrogenase expression modulates longevity and neurodegeneration in *Drosophila melanogaster*. Aging (Albany NY). 2020 Jun 2;12(11):10041-10058. doi: 10.18632/aging.103373. Epub 2020 Jun 2. PMID: 32484787; PMCID: PMC7346061.

13. Newington JT, Harris RA, Cumming RC. Reevaluating Metabolism in Alzheimer's Disease from the Perspective of the Astrocyte-Neuron Lactate Shuttle Model. J Neurodegener Dis. 2013;2013:234572. doi: 10.1155/2013/234572. Epub 2013 Apr 23. PMID: 26316984; PMCID: PMC4437330.

14. Agrawal N, Dasaradhi PV, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK. RNA interference: biology, mechanism, and applications. Microbiol Mol Biol Rev. 2003 Dec;67(4):657-85. doi: 10.1128/MMBR.67.4.657-685.2003. PMID: 14665679; PMCID: PMC309050.

15. Tolwinski NS. Introduction: Drosophila-A Model System for Developmental Biology. J Dev Biol. 2017 Sep 20;5(3):9. doi: 10.3390/jdb5030009. PMID: 29615566; PMCID: PMC5831767.

16. Gostimskaya I. CRISPR-Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. Biochemistry (Mosc). 2022 Aug;87(8):777-788. doi: 10.1134/S0006297922080090. PMID: 36171658; PMCID: PMC9377665.

17. Asmamaw M, Zawdie B. Mechanism and Applications of CRISPR/Cas-9-Mediated Genome Editing. Biologics. 2021 Aug 21;15:353-361. doi: 10.2147/BTT.S326422. PMID: 34456559; PMCID: PMC8388126.

18. Niccoli T, Kerr F, Snoeren I, Fabian D, Aleyakpo B, Ivanov D, Sofola-Adesakin O, Cryar A, Adcott J, Thornton J, Partridge L. Activating transcription factor 4-dependent lactate dehydrogenase activation as a protective response to amyloid beta toxicity. Brain Commun. 2021 Mar 26;3(2):fcab053. doi: 10.1093/braincomms/fcab053. PMID: 33977265; PMCID: PMC8093921.

19. Zhang M, Cheng X, Dang R, Zhang W, Zhang J, Yao Z. Lactate Deficit in an Alzheimer Disease Mouse Model: The Relationship With Neuronal Damage. J Neuropathol Exp Neurol. 2018 Dec 1;77(12):1163-1176. doi: 10.1093/jnen/nly102. PMID: 30383244.

20. Le A, Cooper CR, Gouw AM, Dinavahi R, Maitra A, Deck LM, Royer RE, Vander Jagt DL, Semenza GL, Dang CV. Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. Proc Natl Acad Sci U S A. 2010 Feb 2;107(5):2037-42. doi: 10.1073/pnas.0914433107. Epub 2010 Jan 19. PMID: 20133848; PMCID: PMC2836706.

Figures

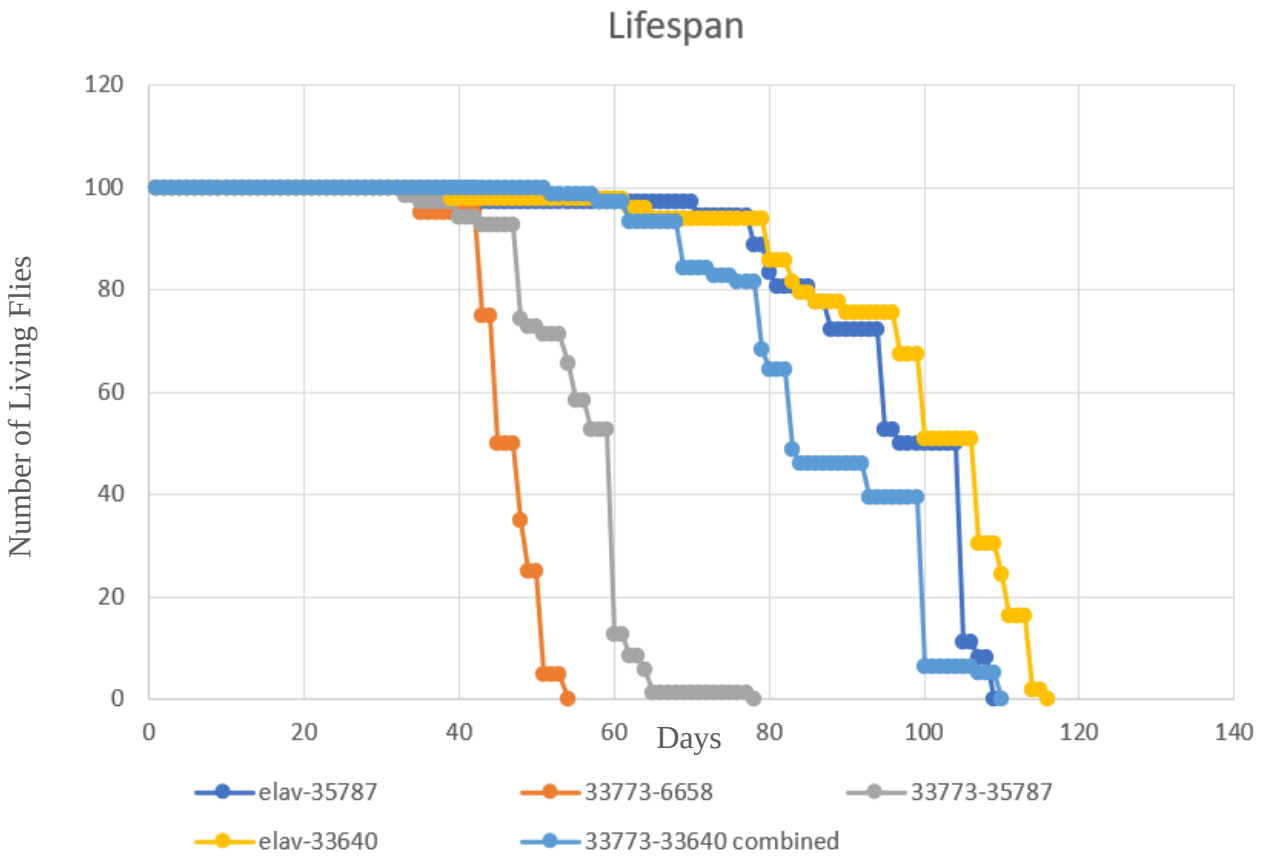

Figure 1. Lifespan of Alzheimer's (AD) flies in the presence of LDH RNAi (elav/33773/33640), compared to that of control (33773/35787)

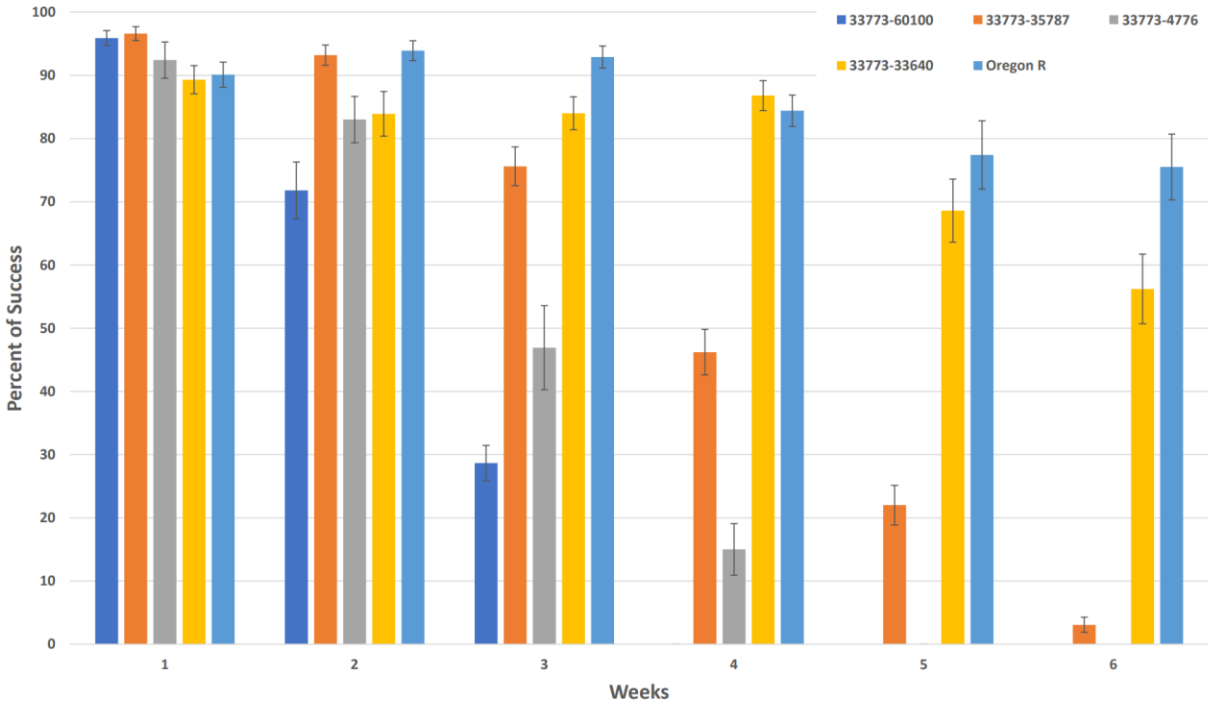

Figure 2. Locomotor function assessed by climbing assay. Knockdown of LDH (yellow; elav/33773/33640) rescued locomotor function, compared to three AD controls, either with vector insertion (elav/33773/60100), with UAS-mCherry (elav/33773/35787), or with UAS-GFP (elav/33773/4776). Oregon Red was included as wild-type control. Data is shown as mean with standard error (SEM)