Retinopathy in Hyperglycemic Zebrafish

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Introduction: Diabetes affects a total of 29.1 million people in the United States of America(nationaldiabetesreport). There are two types of Diabetes: Type 1 and Type II. Type I is caused by a deficiency of Insulin, a hormone secreted by the pancreas (medlineplus). Insulin is used by the body to metabolize glucose, but when the pancreas cannot secrete enough hormone, the sugar builds up in the blood stream resulting in what is called hyperglycemia. In Type II, the pancreas produces insulin, but the body does not respond to it. This also results in an increase in blood sugar. A common symptom of diabetes is retinopathy. This disease causes damage to the tissue in the back of the eye known as the retina. The retina is sensitive to light and creates the images sent to your brain via the optic nerve(Mayo clinic: definition) . Retinopathy ultimately leads to impaired vision(Seeing beyond Retinopathy).

When there is too much sugar in the bloodstream, the small blood vessels which supply the retina with blood can become clogged. In response, the body will attempt to grow new blood vessels, but often they don't mature properly. In the beginning, retinopathy may induce little no symptoms, but as the disease matures there can be serious effects such as blurred vision, fluctuating vision, impaired color vision, dark or empty areas in your vision and even blindness (mayo clinic symptoms). There are two kinds of retinopathy, Non-Prolific (NPDR) and proliferative diabetic retinopathy (PDR). During NPDR, new blood vessels aren't forming, but present blood vessels are leaking fluid and blood causing the retina to swell. PDR is the more serious version of diabetic retinopathy and occurs when new blood vessels grow abnormally into the vitreous. This

can cause scarring, bleeding, and in extreme cases detachment of the retina. Because type 2 diabetes is often diagnosed late, most people already have complications. That being said, retinopathy does not instantly occur once one is diagnosed, but it takes time to develop. This can be excelled by having high blood pressure, smoking, high lipid levels, and pregnancy.

For this experiment, three groups of zebrafish were introduced to three different environments: the first being plain water (stress control), the second being water with glucose in it, and the third being water with mannitol in it (osmotic control). After two and four weeks, a few of the fish were extracted and the optomotor response was tested. This is a visual behavior exhibited by zebrafish where the fish are exposed to an alternating black and white stimulus that is moving. When the whole field is moving, it causes the fish to move in the same direction as the field(behavioral screening assays). A disease like retinopathy would theoretically inhibit the optomotor response in zebrafish. It is hypothesized that hyperglycemia affects this visual behavior in zebrafish through the induction of retinopathy.

Methods: The experiment started with three tanks of 16 fish each. The three tanks were labeled H2O which only contained 2L of water, Mannitol which contained 2L of water and 20 grams of mannitol, and glucose which contained 2L of water and 20 grams of Glucose. These solutions all contained 1% solute. The glucose tank was used to induce hyperglycemia in the fish whereas the H2O tank was used as a control. Mannitol is a sugar that cannot be absorbed by the zebrafish and was used as a control

to make sure that the reason the fish were failing tests was not due to the fact that there was high amounts of solute in the water. Each tank was placed in a water bath that was approximately 26 degrees celsius. Every day the fish would be transported back and forth between their tanks with solutions and tanks with solely water, including the fish that were originally only in water. This was done to ensure that the fish would not become acclimated to the high levels of sugar in their tanks and osmoregulate, or maintain homeostasis between water and solute. In other words, the fluctuation of blood sugar levels of diabetics was emulated throughout the course of 4 weeks. Every day the tanks would be cleaned, the fish would be transferred, the pH would be measured, the temperature of the water bath would be recorded and the tanks for the next day would be made. On top of this, every other day the fish would be fed tetramin (fish food). After two weeks, about 4-5 fish were taken out of each tank and their optomotor response was tested. This was conducted by placing them in a small flat-bottom bowl in a dark cabinet. Under the bowl was a computer screen that ran a visual display under the fish. This visual was a large wheel with 12 wedges and turned at 1.04 rotations per second.It started by turning clockwise for 30 seconds, white for 30 seconds, turned counter clockwise for 30 seconds, then white again for 30 seconds. This was repeated twice for each group of fish and recorded with a camera. Afterwards the videos were analyzed with two different methods, scatter sampling and total rotations. For scattering sampling, every 6 seconds, if the fish were swimming with the stimulus, it was recorded. For example if one of the fish was swimming in the right direction I would write 1, if two, 2, if none,0. For total rotations, the total number of times a fish travels completely around the

wheel in the direction of the visual stimulus is recorded. After the behavioral trials are completed, the fish are anesthetized, weighed, tested for blood sugar levels, and retinal and brain tissue were removed and flash frozen for later analysis. The retinas were taken out so that it could be determined whether or not retinopathy had taken place. After the two week trial, the amount of solute in each tank was increased from 20g to 40g increasing the percent of solute from 1% to 2%. After another 2 weeks, or 4 weeks in total, the final fish underwent these same procedures.

Results: All of the graphs use averages and compare these averages from week 2 to week 4. In graph 1, all of the blood glucose levels were taken from each fish and averaged together. This was done using a blood glucose monitor. As seen in graph 1 , the blood glucose levels were close across all treatments after two weeks of treatment; staying within the 30-60 mg/dL. But, after 4 weeks of treatment the blood glucose levels of the fish being treated with glucose increased drastically while the levels for the other tanks fell. A similar trend can be seen in graph 2 when comparing weights. The average weights after week 2 were all similar but after 4 weeks the average weight of the glucose fish more than doubled whereas the average weights of the other fish decreased. This was not expected because hyperglycemia results in a decrease in weight instead of an increase. The weights in graph 2 were all found by weighing each fish individually from each treatment and averaging them together. Graph 3 and 4 pertain to the two behavioral tests: scatter sampling and total rotations. In graph 3: total rotations, the total number of rotations was added together from each tank. Then the

total number was divided by the number of fish from each tank that was tested. After 2 weeks, the fish in the glucose tank had completed less total rotations than all of the other fish. After 4 weeks the two remaining fish from the glucose couldn't complete a single full rotation along with the one remaining fish from the mannitol tank. Graph 4 displays the data gathered from the scatter sampling. This was done by adding up the number of positive observations (the number of fish swimming the right direction every 6 seconds) and divided by the number of fish from each tank. The data gathered for graph 4 shows that the fish in the mannitol tank had the most difficult time following the stimuli having the lowest numbers after both 2 and 4 weeks. When the lenses were dissected from each fish, the only ones that were cloudy were taken from the final two glucose fish after 4 weeks of treatment.

Discussion: It was expected that the blood glucose levels of the fish treated with glucose to rise, the weight to decrease, the lenses to be clouded, and the results of the scatter and total rotations tests to be lower than those of the fish treated in H2O and mannitol. Because of the data shown in graph 1, it can be safely assumed that the fish in the glucose treatment underwent hyperglycemia as planned. As seen in graph 2, the weight of the glucose fish skyrocketed when comparing week 2 to week 4. This is unexpected because hyperglycemia causes subjects to lose weight. Weight loss occurs because when there is too much sugar in the bloodstream, the body works extra hard burning calories and fat in an attempt to digest all of the sugar (Diabetes.uk). This may have been due to improper food distribution. In terms of optomotor response testing, the results were rather inconclusive because the total rotations and scatter sampling of the

glucose fish mirrored those of the mannitol fish. In both behavioral tests, by week four neither group could complete a single rotation nor could either score well on the scatter sampling test. This is due to the fact that the osmolarity of the mannitol and the glucose tanks was too high and caused the impairments of the optomotor response. This is evident because the H2O fish score much higher on both the scatter sampling and total rotations tests. It is also worth mentioning that only one of the fish had a slightly clouded lens and it was from the glucose tank, therefore leading us to believe that only one of the fish was starting to develop retinopathy. In order to further test this hypothesis the trial length would need to be increased either to 6 or 8 weeks, allowing retinopathy to more fully develop. Another source of error could have stemmed from the fact that there was only one fish from the mannitol tank left and two fish left from the glucose tank. This sample size was rather small and could have contributed to the errors of the experiment.

Sources:

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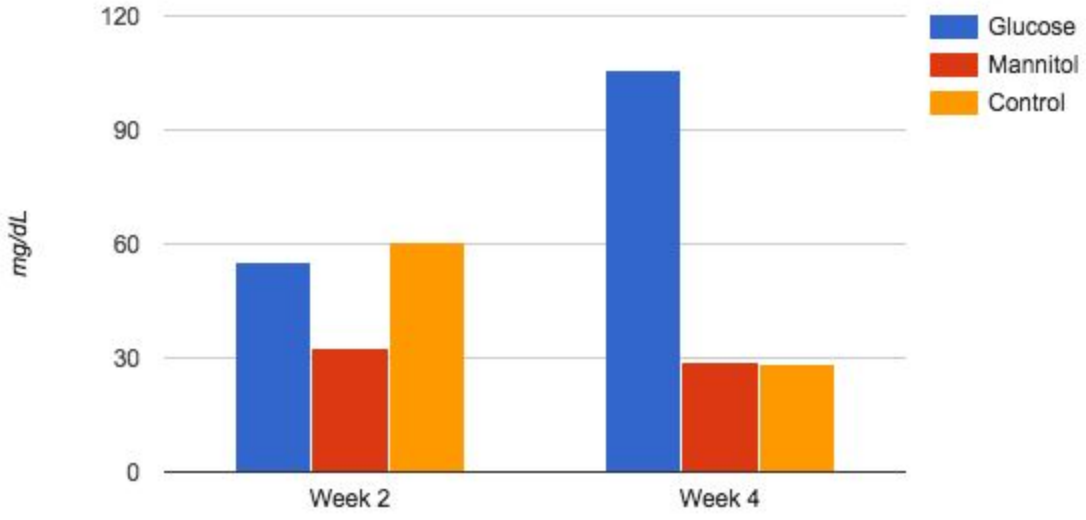
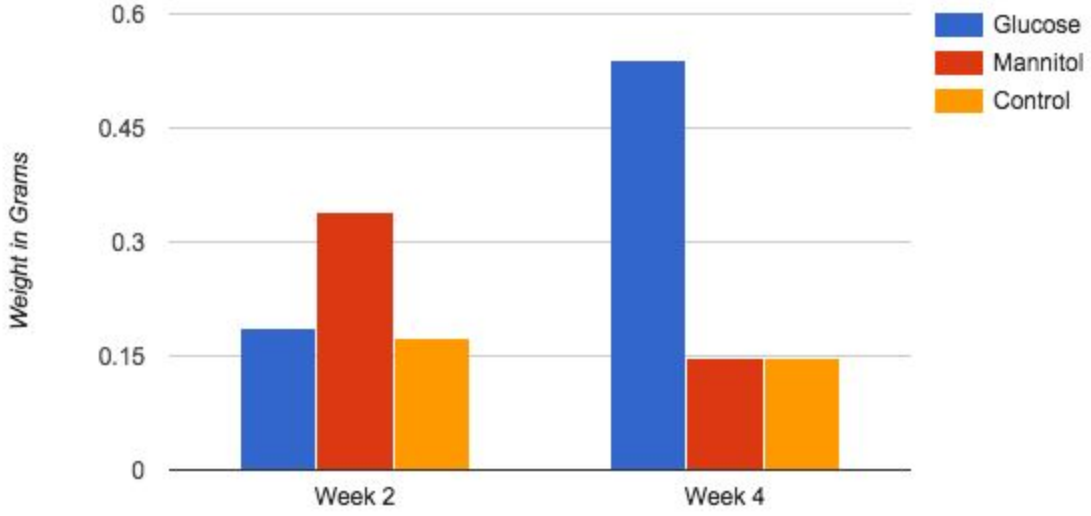
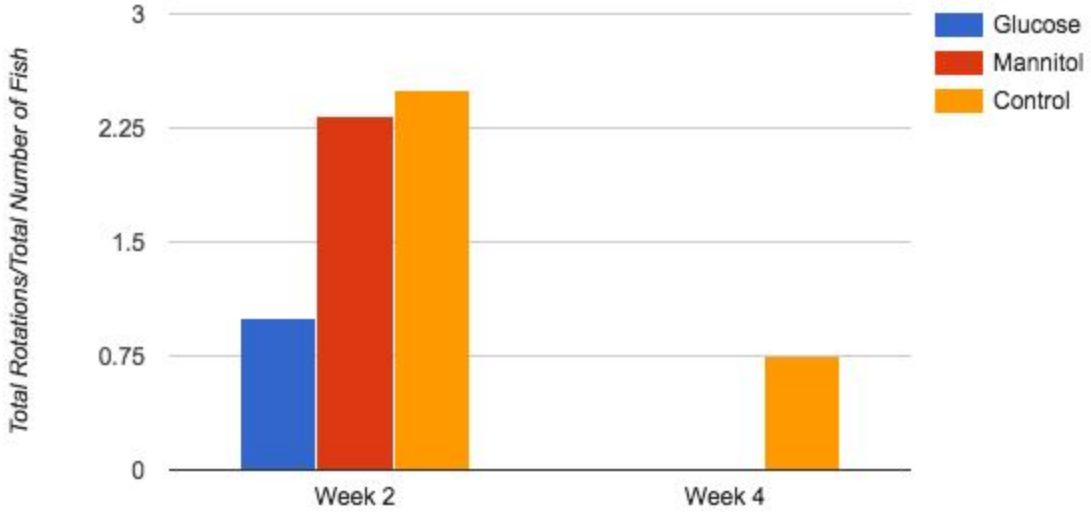
Graph 1: Average Blood Glucose Levels Between Week 2 and 4

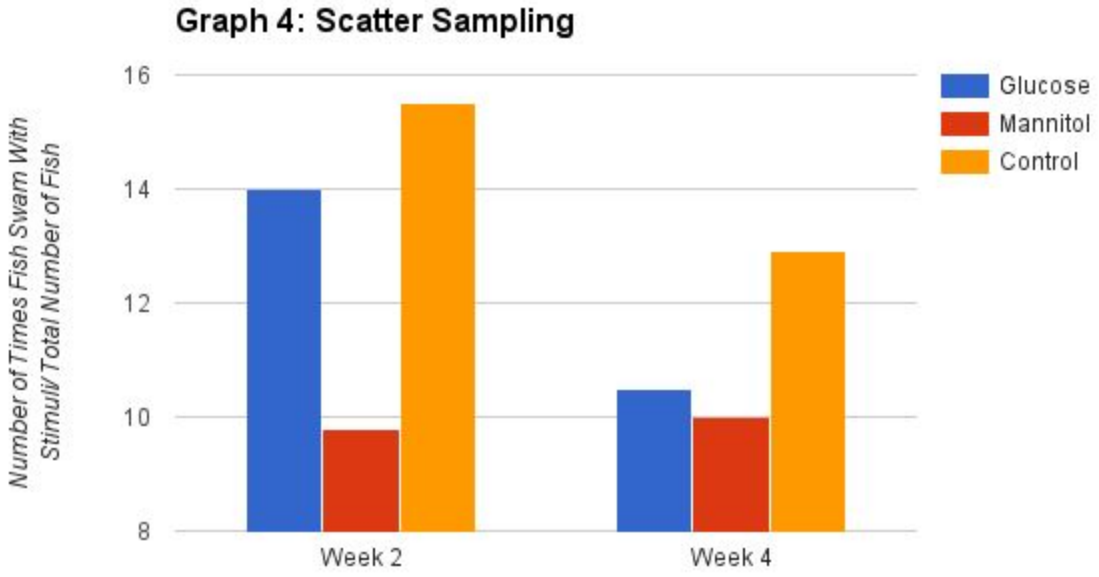


Graph 2: Average Weight Between Week 2 and 4



Graph 3: Total Rotations




Graph 4: Scatter Sampling
Number of Times Fish Swam With Stimuli/ Total Number of Fish
16
14
12
10
8
Week 2
Week 4
Glucose
Mannitol
Control