The Effects of Glucose on the Embryological Development of Zebrafish
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ABSTRACT  The purpose of this experiment was to investigate and demonstrate the negative effects that glucose has on embryological development. As a representation of a human embryo, zebrafish embryos were used. It was found that glucose exposure to zebrafish embryos can cause accelerated heart rate, obesity and even death. The zebrafish embryos’ exposure to the different glucose solutions reciprocated the indirect exposure that a human embryo would experience through its mother during pregnancy.

INTRODUCTION  In the United States, more than one-third of children or adolescents aged 6-19 are considered to be overweight or obese. Obesity can lead to type 2 diabetes, heart disease, high blood pressure, stroke, certain types of cancer and often death, and is caused by excessive amounts of body fat as a result of consuming high amounts of sugar. This is not limited to the eating habits of the person, but also includes what the mother eats during pregnancy. Glucose is the common form of sugar, which was the toxicant used in this experiment.

In this experiment, zebrafish were used to model the effects of glucose on developing babies that are exposed to high concentrations of glucose from their mothers during pregnancy. Zebrafish are common household pets that can also be used as model organisms to study the effects of common chemicals in the environment on embryological development. Zebrafish are exceptional models due to the fact that they develop outside of the mother, are produced in large quantities, exhibit synchronous development with other zebrafish in a clutch, are visually clear, and develop rapidly.

The variable that was tested in this experiment was glucose. The hypothesis in this experiment was that exposure to a lower concentration of glucose (0.05%) would cause developmental problems in embryos such as an accelerated heartbeat and that exposure to a higher concentration of glucose (0.5%) would cause more drastic developmental problems including accelerated heartbeat and obesity.

MATERIALS AND METHODS  The materials used in this experiment included a well plate that was sectioned into three separate rows with four wells in each row, a small bore and a large bore disposable pipette plus extras, solutions of glucose (0.05% and 0.5%), a 100 mL waste beaker, Instant Ocean/Embryo Media Solution, and both an optical microscope and a stereoscope. All of the materials were provided by the Wisconsin Inquiry-based Scientist-Teacher Education Partnership (WInSTEP) Program, which is part of the NIH Science Education Partnership Award (SEPA) Program administered by the University of Wisconsin–Milwaukee and the Children's Environmental Health Sciences Core Center.

First, 10 living zebrafish embryos were carefully placed into each well using a disposable pipette. The Embryo Media Solution was then added to each well in row A, also known as the control row, in row B, 0.05% glucose solution was added, and in row C, 0.5% glucose solution was added. The embryos were carefully examined using a microscope and notes were taken regarding the state of development. The next day, the number of living embryos or hatched embryos were recorded and the Embryo Media Solution or the glucose solution was changed. Any dead embryos were discarded into a separate beaker to be disposed. This process was
repeated each day for a total of 4 days. Gloves and safety goggles were worn when handling the glucose solutions. After the experiment was conducted and the data was collected, an online statistical t-test was used to determine whether the data had a correlation with the hypothesis. The website used was [http://www.graphpad.com/quickcalcs/ttest1/](http://www.graphpad.com/quickcalcs/ttest1/)

**RESULTS** The independent variable in the experiment was the glucose solution or the control and the dependent variables were the number of living and hatched zebrafish. The control of the group was row A which contained only the Embryo Media Solution, row B contained a 0.05% glucose solution, and row C contained a 0.5% glucose solution. After the experiment was concluded, the final data was analyzed using a statistical t-test to show that high concentrations of glucose have a direct correlation with mortality amongst zebrafish embryos. There were no abnormalities with mortality rates in the control and row B, but the zebrafish fry in row B had enlarged yolk sacs, as shown in **Figure 1**. When observing the heart rate of the living zebrafish, the zebrafish in row B had a more rapid heartbeat than those in row A.

![Figure 1](image1.png)

**Figure 1** On the left is a picture of a hatched zebrafish that was exposed to the 0.05% glucose solution, or the low concentration. On the right is a healthy zebrafish from the control group that was not contaminated with any glucose solution. The yolk sac of the zebrafish on the left is significantly larger than the one on the right.

In row C, all of the embryos were deceased by the end of the experiment. According to the statistical t-test that was done on the data, it can be proven that there is a significant correlation between the data and the hypothesis, meaning that the zebrafish died as a result of the high concentration of glucose. **Figure 2** demonstrates what the dead embryos in row C looked like. Looking at the dead embryos with the naked eye, they were white in color.

All the data that was recorded of the living and hatched zebrafish are included in **Table 1** and **Table 2**, and **Figure 3** and **Figure 4** shown below.
**Figure 2** This is a picture of a dead embryo that was found in row C, contaminated with the 0.5% glucose solution. All of the embryos found in this row looked just as this single embryo does.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Well 1</th>
<th>Well 2</th>
<th>Well 3</th>
<th>Well 4</th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Probability</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>8</td>
<td>9</td>
<td>9</td>
<td>7.75</td>
<td>1.892</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05%</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>1.154</td>
<td>0.3026</td>
<td>Not Significant</td>
</tr>
<tr>
<td>0.5%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0002</td>
<td>Extreme</td>
<td>Extremely Significant</td>
</tr>
</tbody>
</table>

**Table 1** This table shows the final number of living fry for each well along with the average living fry for each concentration of glucose and the control. This also shows the standard deviation and the significance of the data in regards to the hypothesis. The data shown in this table is represented in **Figure 3**.
Figure 3 This figure shows the final number of live fry along with the average living fry for each concentration of glucose and the control. There is a very low mortality rate in the control and the 0.05% concentration and a high mortality rate in the 0.50% concentration.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Well 1</th>
<th>Well 2</th>
<th>Well 3</th>
<th>Well 4</th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Probability</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>1.5</td>
<td></td>
<td></td>
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<tr>
<td>0.05%</td>
<td>8</td>
<td>10</td>
<td>9</td>
<td>8</td>
<td>8.75</td>
<td>0.957</td>
<td>0.0656</td>
<td>Not Significant</td>
</tr>
<tr>
<td>0.5%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0001</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Table 2 This table shows the final number of hatched zebrafish along with the average number of hatched fry per concentration of glucose and the control. This table also shows the standard deviation and the significance of the data in regards to the hypothesis. This data in this table was used in making Figure 4.
Figure 4 This figure shows the final number of hatched zebrafish along with the average number of hatched fry per concentration of glucose and the control. The majority, but not all, of the zebrafish embryos were hatched by the end of the experiment in rows A and B, and none of the embryos in row C hatched due to the fact that they all died.

DISCUSSION The data collected throughout this experiment partially supported the hypothesis, and partially did not. It was hypothesised that zebrafish embryos that were exposed to the higher concentration of glucose would have serious developmental problems, but the embryos showed no sign of developmental defects and instead, died. The zebrafish embryos in the lower glucose concentration were thought to express less severe developmental flaws such as accelerated heart rate, which stood to be true. In the beginning, it was unknown of whether the embryos exposed to the 0.05% glucose solution would show signs of obesity, due to the fact that the concentration is so low, but experiment showed that the embryos exposed to the low concentration of glucose had enlarged yolk sacs, which could potentially be related to obesity. According to the statistical t-test that was done, the mortality rates shown in row C of both tables were significant, which means that the zebrafish embryos died as a result of such a high concentration of glucose that they were exposed to.
There were many potentials for errors in this experiment. At the beginning of the experiment, 10 zebrafish embryos were to be put in the separate wells of the well plate. The embryos are very tiny, and almost transparent, thus making it difficult to be counted perfectly using only the naked eye. While conducting solution changes within the wells, some embryos were unaccounted for, meaning that the original counting was incorrect, or the embryos were sucked up in the pipette and disposed of, unknowingly. There were some deaths amongst the embryos in the control group, or row A. This could possibly be due to contamination by using a pipette in a well containing one of the glucose solutions in them and then using it in the control row, but they could very well have just died of natural causes. Since the experiment was only conducted within 4 days, there is no way to tell if there could have been any future effects to the living zebrafish within row B.

The experiment conducted can be used as a model to show how glucose intake of a woman during pregnancy can affect the baby immediately, or some time after giving birth. High fructose corn syrup, which is commonly found in most processed foods, is broken down by the body into glucose, which is then stored in the body. When there are large amounts of glucose in the body, it creates large amounts of unnecessary body fat. Having an excess of body fat, also known as being overweight or obese, has many consequences to a human’s health, including heart disease. If a pregnant woman consumes high amounts of high fructose corn syrup, her baby is at risk of heart disease and obesity because of that indirect exposure to the high glucose levels. This experiment can help people to understand the negative effects on embryological development that occur when a mother consumes a diet that contains high amounts of sugar.

REFERENCES