Abstract

The purpose of this experiment was to test the effects of sodium chloride (table salt) on the embryonic development of zebrafish. Zebrafish were chosen for the experiment because they are model organisms, meaning that the knowledge gained from this experiment can be used to better understand the effects of sodium on human embryos. The majority of Americans consume more than the recommended amount of sodium, resulting in a slew of health complications related to a high sodium diet in the United States. With the health concerns surrounding a high sodium diet the question of whether sodium has an affect on developing human embryos is raised. In the experiment, zebrafish embryos were exposed to embryos media solution, 240 mg/L saline solution, 480 mg/L saline solution, and 1000 mg/L saline solution for four days. Each day, dead embryos were removed and data and observations were recorded as the solutions were replaced. The results of the experiment showed that sodium has little to no effect on the embryonic development of zebrafish. From the results of the experiment it can be inferred that sodium has little to no affect on the development of human embryos, but further studies would need to be done.

Introduction

Zebrafish (*Danio rerio*) is a tropical freshwater fish belonging to the minnow family that is frequently sold for use in aquariums. However, what makes zebrafish important to researchers is for their use in understanding the basic principles of vertebrate development. Zebrafish are an important and widely-used model organism used in scientific studies. Model organisms, such as zebrafish, are species that have been widely studied because they have particular experimental advantages. A variety of characteristics of zebrafish embryos make them impressive models for scientific research. Zebrafish embryos develop outside of the mother and are optically clear which allows researchers to easily view and study the embryos. Zebrafish are produced in large numbers and exhibit synchronous development within a clutch, which allows researchers to study large numbers of individuals at the same time. Zebrafish also develop rapidly, so researchers are able to complete trials in a one week time block, which is a relatively short period of time. Zebrafish are also intriguing to researchers because researchers can predict the effect of a certain substance on humans from the results of zebrafish experiments. This can be done partly because zebrafish share 70% of their genetic code with humans (McKie, 2013).

The development of zebrafish and other vertebrate can be divided roughly into three phases that include an initial early phase of rapid cell divisions, the establishment of the three body axes, and the development of organ systems. In zebrafish, rapid cell divisions occur over the first 2-3 hours after fertilization, with the first cell division occurring within about 0.5 hours.
after fertilization and later cell division occurring approximately every 15 minutes. From approximately 3 to 10 hours after fertilization, the development of the zebrafish embryos results in the creation of three body axes: anterior-posterior, left-right, and dorsal-ventral. Zebrafish embryos enter the high dome stage and begin to undergo the process of epiboly after about 4 hours post fertilization. During epiboly, cells move down the sides of the yolk until until a layer of cells covers the entire yolk of the fish egg. Cells at the advancing edge begin to move underneath the cell cell late in epiboly. This process is known as gastrulation, which occurs approximately 5.25 to 10 hours after fertilization. The result of gastrulation is the formation of three distinct primary cell or germ layers known as endoderm, mesoderm, and ectoderm. These cell or germ layers will give rise to all of the tissues found in the adult fish. By the end of epiboly, after about 9 hours post fertilization, the anterior-posterior (head to tail) axis will be visible. The process of gastrulation is followed by the formation of somites during the period of segmentation, which occurs 10 to 24 hours after fertilization. The formation of somites causes muscle precursors and nervous system development. Somites are the visible and chevron-shaped structures in the posterior portion of the embryo. By 13 hours after fertilization, the precursor to the eye is evident. By 25 hours after fertilization the developing eye is visible, the developing brain and otic vesicle of the ear is apparent, the heart begins beating, and the yolk of the egg is completely incorporated into the body of the embryo. From 48 to 72 hours after fertilization, the fish are hatching and beginning to swim.

Toxicology is the quantitative and qualitative study of the adverse effects of toxicants on biological organisms. Sodium is a toxicant, or a chemical or physical agent that causes adverse effects in biological organisms, that is readily consumed by the American public in large proportions. The 2010 Dietary Guidelines for Americans recommend an upper limit for sodium consumption of 2,300 milligrams of sodium per day for adults. However, 90% of Americans consume more sodium than what is recommended for a healthy diet. 75% of this sodium comes from either processed foods or restaurant foods (Ruggeiro, 2015). A diet that is too high in sodium can have many severe health effects in humans. A high salt intake can lead to high blood pressure, which is a risk factor for stroke, heart disease, kidney disease, and congestive heart failure. A diet high in sodium is also known to lead to cognitive decline, decreased kidney function, bone thinning, and edema (swelling, particularly in the arms, hands, ankles, legs, and feet) (Dovey, 2015).

Among the Americans who consume more sodium than what is recommended are expectant mothers. With all the health concerns surrounding a high sodium diet an interesting question can be raised. Does an expectant mother’s sodium intake affect the development of the embryo she carries? To help answer this question the development of zebrafish embryos can be studied in various saline solutions. From this research, a prediction can be made about the effects of sodium on human embryos. Will the development of zebrafish embryos be affected by sodium? If zebrafish embryos are exposed to high concentrations of sodium, then the zebrafish embryos and fry will be greatly affected. The embryos exposed to high concentrations of sodium
may develop more slowly, develop shortened tails, have decreased kidney function, have high blood pressure, lose cognitive function, developed smaller eyes, and develop swollen and deformed skin tissue.

Materials and Methods

The materials used in this experiment included one bottle of 240 mg/L saline solution, one bottle of 480 mg/L saline solution, one bottle of 1000 mg/L saline solution, one 250 mg/L beaker of embryo media solution, one 100 mL beaker for dead embryos and liquid disposal, one Expo marker, large bore disposable pipettes, small bore disposable pipette, one plate with wells, one 28.5°C incubator, one dissecting and compound microscope, and 120 zebrafish embryos. 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.

On day 1, the spawning tank was set up and the feeding of the brine shrimp in preparation for spawning was completed. On day 2, rinsed embryos were obtained from the instructor. The concentrations on each well was labeled appropriately. One row of the well plate was then filled with 1 mL of embryo media solution using the disposable pipette. The remaining rows of wells were filled with the appropriate saline solution (240 mg/L, 480 mg/L, and 1000 mg/L). Gloves and goggles were worn whenever work was done with sodium solutions. The embryos were divided so that 10 embryos were placed in each well. Finally, the plate was placed in the 28.5°C incubator overnight. On day 3, the plate was removed from the incubator. The dead embryos were removed from the well plate using a disposable pipette. The dead embryos were then squirted into the waste beaker. Then the remaining embryos and hatched fish were counted and this value was recorded in the data table. The liquid was then removed from each well of the plate using a disposable pipette. Next, the wells were refilled with their appropriate solution with a disposable pipette. After this, the plate was placed under a microscope and each well was carefully examine. Observations were recorded. Finally the plate was returned to the 28.5°C incubator. On day 4, the day 3 work was repeated. Next, the embryos were disposed of.

Once the lab was complete, the hatch rate and mortality rate values of the control were compared to the hatch rate and mortality rate values of each treatment through a GraphPad Software t-test to determine the significance of the results of each treatment.

Results

The majority of Americans consume more sodium than what is recommended. A high sodium diet can have devastating health consequences in humans. With the plethora of health concerns surrounding a high sodium diet one may wonder what affects a high sodium diet of an expecting mother has on the developing embryo inside her. The goal of the experiment was to determine if a high sodium concentration causes complications in developing zebrafish embryos.
so a prediction can be made about the effects of sodium on developing human embryos. Sodium was the independent variable in the experiment. Mortality rate and hatch rate were the dependant variables. The results of results of the experiment did not support the hypothesis and showed that sodium has little to no effect on the embryonic development of zebrafish. The results of all three saline solutions (240 mg/L, 480 mg/L, and 1000 mg/L) proved to be insignificant. The control of the experiment was wells (A1, B1, and C1) in which zebrafish embryos were exposed to embryo media solution. If zebrafish embryos are exposed to high concentrations of sodium, then the zebrafish embryos will be greatly affected.

Table 1: Surviving Zebrafish Fry by Treatment on Day 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Well 1</th>
<th>Well 2</th>
<th>Well 3</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>5</td>
<td>3</td>
<td>4.33</td>
<td>1.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>240 mg/L</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>6</td>
<td>1.73</td>
<td>p=0.24</td>
<td>Not Statistically Significant</td>
</tr>
<tr>
<td>480 mg/L</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>6.33</td>
<td>1.15</td>
<td>p=0.10</td>
<td>Not Statistically Significant</td>
</tr>
<tr>
<td>1000 mg/L</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>5.67</td>
<td>1.15</td>
<td>p=0.23</td>
<td>Not Statistically Significant</td>
</tr>
</tbody>
</table>

The table shows the number of surviving zebrafish fry on day 4 of the experimental study. The number of surviving zebrafish fry are listed by well of each treatment. An average of the number of surviving zebrafish fry for each treatment is shown on the table. Standard deviation values for each treatment are also shown in the table. Probability values and result from a t-test shown for each of the saline solutions.

Figure 1: Average Number of Surviving Zebrafish Fry by Treatment on Day 4

The figure shows the average number of surviving zebrafish fry by treatment on day 4 of the experimental study. Standard deviation values are also represented on the graph.
Table 2: Hatched Zebrafish Fry by Treatment on Day 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Well 1</th>
<th>Well 2</th>
<th>Well 3</th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Probability</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>5</td>
<td>3</td>
<td>4.33</td>
<td>1.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>240 mg/L</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>5.33</td>
<td>0.58</td>
<td>p=0.25</td>
<td>Not Statistically Significant</td>
</tr>
<tr>
<td>480 mg/L</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td>p=0.49</td>
<td>Not Statistically Significant</td>
</tr>
<tr>
<td>1000 mg/L</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>4.67</td>
<td>1.15</td>
<td>p=0.74</td>
<td>Not Statistically Significant</td>
</tr>
</tbody>
</table>

The table shows the number of hatched zebrafish fry on day 4 of the experimental study. The number of hatched zebrafish fry are listed by well of each treatment. An average of the number of hatched zebrafish fry for each treatment is shown on the table. Standard deviation values for each treatment are also shown in the table. Probability values and result from a t-test shown for each of the saline solutions.

Figure 2: Average Number of Hatched Zebrafish Fry by Treatment on Day 4

The figure shows the average number of hatched zebrafish fry by treatment on day 4 of the experimental study. Standard deviation values are also represented on the graph.
Discussion

The results of the experiment did not support the hypothesis that if zebrafish embryos were exposed to high concentrations of sodium, then the zebrafish embryos and fry would be greatly affected. Instead the results of the experiment supported the opposite conclusion. The results of the experiment supported the idea that sodium has little to no affect on the development of zebrafish embryos. The zebrafish embryos exposed to high concentrations of sodium developed few of the health complications they were predicted to. Although it was observed that two zebrafish fry that were exposed to 480 mg/L saline solution in well B3 and one zebrafish fry that was exposed to 480 mg/L saline solution in well C3 did develop short curved tails and an irregular swimming pattern on day 4 of the experimental study, a similar
phenomenon was also observed in well 2 of the control on day 4 of the experimental study as well. Other than this tail deformity, no other deformities were observed in any of the treatment or control wells. The average amount of hatched and surviving zebrafish was fairly consistent across all treatments and the control. All averages were within 2 embryos or fry of the control. The probability values for both the amounts of surviving zebrafish fry and hatched zebrafish fry were insignificant for all three of the treatments (240 mg/L, 480 mg/L, and 1000 mg/L).

Errors made in the experiment may have affected the accuracy of the results. Some zebrafish embryos were accidently removed from the wells as solution changes were completed. On day 2 of the experimental study, one embryo was accidently removed from well A1, one embryo was accidently removed from well A2, two embryos were accidently removed from well A3, and one embryo was accidently removed from well B4. On day 3 of the experimental study, one embryos was accidently removed from well B4. Although these embryos were excluded from the data they certainly had an affect on the results of the experiment. While doing solution changes, the same disposable pipettes may have been used in well of different solutions, changing the actual concentrations within these wells. Also while doing solution changes wells may have been filled with the wrong solution. Healthy embryos and fry may have been mistaken for dead ones and removed. The amount of sodium or water may have been measured incorrectly while preparing the solutions. Several limitations also limited the experimental study. As a result of the amount of embryos that were available only 10 embryos could be tested in each well. As a result of time limitations, the zebrafish could not be studied into adulthood.

Based on experimentation with zebrafish embryos a high sodium diet probably has a rather small effect on developing human embryos. However, further experimentation would have to be done on human embryos. Although zebrafish are model organisms, human embryos may still react differently to high sodium concentrations.

References

