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The Effects of Ethanol Exposure on Glucose and Lactate Levels in Crayfish

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The Effects of Ethanol Exposure on Glucose and Lactate Levels in Crayfish

Mackenzie Kirwan

Department of Biology

Honors Research Project

Submitted to
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The University of Akron

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Introduction

The overall objective of this research experiment was to investigate the physiological response of crayfish to acute ethanol exposure. I investigated the response of hemolymph glucose and lactate to ethanol exposure and exercise. Glucose and lactate have crucial roles in exertion and motor function; muscles use glucose during physical activity, while lactate is produced during anaerobic exercise by contributing to the production of ATP (Sandoo, 2021). Knowing that energy is exhausted during exercise, and such energy is used in the process of producing and metabolizing lactate, increasing levels of lactate after exercise are common. Therefore, following anaerobic activities, lactate accumulates due to an increased need for ATP (Sandoo, 2021).

Although glucose is an energy source, it also has high concentrations during or following high stress events. When the body perceives stress, it signals to increase glucose production or decrease glucose uptake as a preservation or survival mechanism (Nirupama, 2018). Crayfish exposed to a stress-induced environment experience higher titers of glucose in their hemolymph (Caldari-Torres, 2018). Exposure to ethanol creates stressful conditions for crayfish. Ethanol is an alcohol and is an inhibitor of excitatory neurotransmitters, which can contribute to decreased function and motor movement (Valenzuela, 1997). A similar study that analyzed behavioral effects after ethanol exposure in crayfish reported decreasing running distance and speed compared to normal water conditions (Gutierrez, 2022).

In stressful environments, cortisol, a stress related hormone, is released which can lead to increase in serum glucose and lactate (Zhu, 2023). When the liver processes and metabolizes alcohols, serum lactate levels also increase (Wilson, 2020). Given the role of ethanol as a promoter of stressful conditions, it was expected that hemolymph glucose and lactate levels would rise following ethanol treatment, while glucose levels would decrease and lactate would continue to increase following exercise to exhaustion.

Materials and Methods
Thirty *Procambarus clarkii* were obtained from the University of Akron Biology department and randomly divided into three subject groups, each consisting of 10 individual crayfish. All crayfish underwent equal experimentation and were also subject to a similar study that focused on metabolic rate and rate of exhaustion. The first was a control group, exposed to aerated water throughout the experiment; the second group was exposed to aerated water with 1% Ethanol and the third group was placed in aerated water with 5% Ethanol. Prior to experiments, crayfish resided in large tanks, with four to five animals in each. Nova Max Plus Blood Glucose Monitoring System and Arkray Lactate Pro Blood Lactate Test Meter kits were used to measure hemolymph glucose and lactate levels, respectively. Monitors and strips were included in these kits. Using a needle and syringe, approximately 0.2mL of hemolymph was extracted from the ventral side of the crayfish and its lactate and glucose levels were measured.

Hemolymph lactate and glucose levels were recorded three times for each crayfish. The first extraction of hemolymph was an initial baseline recording of glucose and lactate prior to experimental exposure and exercise. Depending on what group each crayfish was randomly selected for, crayfish were transferred to individual tanks for two hours after the initial glucose and lactate measurement. For the control group, individual tanks were filled with 1 liter of aerated water. For the group exposed to 1% ethanol, individual tanks were filled with 990 milliliters of aerated water and 10 milliliters of 100% ethanol solution. The group exposed to 5% ethanol were placed in individual tanks with 950 milliliters of aerated water and 50 milliliters of 100% ethanol solution. Each individual tank was sealed with parafilm following placement of the crayfish and a release of air to ensure that there were no air bubbles in the tank and no external air could further oxygenate the water. While undergoing treatment, crayfish were left in their respective tanks for two hours.

Following approximately two hours of the solutions of water and ethanol exposure, glucose and lactate levels were measured, following the same steps as the initial measurement. Crayfish were transferred to a large tank and exercised until exhaustion. To do so, crayfish were chased with a small handheld net. Exhaustion was determined by observing a behavior that rejects aggressive behavior towards the net and retreats from fast movements, such as tailing flipping, when prompted. The final
recording of glucose and lactate were measured after the crayfish demonstrated signs of exhaustion. Upon completion of the experiment, individual weights of each crayfish utilized in this experiment were measured and recorded. Crayfish were placed back into original large tanks after all values were recorded. Using the three recorded values of glucose and lactate under three different experimental conditions, further interpretation of the data was analyzed using a paired two-sample means t-test. These tests are most useful when interested in measurements under different conditions within the same group. P-values were calculated from the paired t-tests and standard deviations were determined from the means. One set of t-tests compared the pre-exposure and post-exposure measurements within each group, then the post-exposure and post-exhaustion within each group. The other set of t-tests compared the change in glucose and change in lactate from pre-exposure to post-exposure, then post-exposure to post-exhaustion between the different treatment groups.

Results

The following results derive from analyzing the change in lactate and glucose levels within each treatment group, the control, 1% ethanol exposure and 5% ethanol exposure. Glucose remained constant for the control group and 1% ethanol exposure, yet wavered for the 5% ethanol group, as levels increased then decreased. For every treatment group, lactate levels increased with each measurement.

For the control group, average glucose levels remained constant at 10 mg/dL ± 0 for each measurement (Figure 1). The average lactate levels for the control group increased with all measurements from the pre-exposure to post-exposure to post-exhaustion, measuring 0.45mmol/L ± 0.16, 1.55mmol/L ± 2.31, and 2.89 mmol/L ± 1.76, respectively (Figure 2). The 1% ethanol exposure group also maintained the same consistent average glucose levels of 10 mg/dL ± 0 for all three measurements (Figure 1). The lactate levels for the 1% ethanol exposure group increased with all measurements from the pre-exposure to post-exposure to post-exhaustion, measuring 0.59mmol/L ± 0.34, 1.17mmol/L ± 0.65, and 2.10mmol/L ± 1.80, respectively (Figure 2). For the 5% ethanol exposure group, glucose levels increased from the pre-exposure at 10 mg/dL ± 0 to post-exposure at 11.9 mg/L ± 6.01, then decreased to 11.6 mg/dL ± 5.06 (Figure 1). The lactate levels for the 5% ethanol exposure followed the same trend as other treatments
group by increasing with all measurements from the pre-exposure to post-exposure to post-exhaustion, measuring $0.44 \text{mmol/L} \pm 0.13$, $2.26 \text{mmol/L} \pm 1.28$, and $3.33 \text{mmol/L} \pm 2.31$, respectively (Figure 2).

From the set of t-tests that compared the changes in glucose and lactate levels from the pre-exposure to post-exposure and post-exposure to post-exhaustion between the different treatment groups, one test was statistically significant. All other p-values calculated from the statistical tests had a value greater than 0.05. The p-value that was statistically significant was 0.004 compared the change in lactate levels from the pre-exposure to post-treatment measurements between the control group and 5% ethanol exposure group (Table 1).

From the t-tests that compared the measurements of pre-exposure to post-exposure and post-exposure to post-exhaustion within each group had three p-values deemed to be significant. The control group had no significant differences. The 1% ethanol exposure group demonstrated a statistical significance in the t-test that compared pre-exposure to post-exposure lactate levels, with a p-value of 0.002 (Table 2). The 5% ethanol exposure group demonstrated a statistical significance in both t-tests for lactate; the test that compared the pre-exposure to post-exposure yielded a p-value of 0.0008 and the test that compared the post-exposure to post-exhaustion yielded a p-value of 0.05 (Table 2).

**Table 1. P-Values for Change in Glucose and Lactate** below presents the p-values calculated from each t-test that compared the changes between the control group to the 1% ethanol and the control group to the 5% ethanol group in glucose and lactate levels from the pre-exposure to post-treatment and from post-treatment to post-exhaustion. The p-value labeled with an asterisk (*) symbol is statistically significant. A p-value with numeric value less than 0.05 represents data that is replicable and rejects a null hypothesis, therefore, is statistically significant. Of all data collected, the comparison of lactate changes between the control group and group exposed to 5% ethanol is the only relationship that yields a statistically significant result.

<table>
<thead>
<tr>
<th>T Test P-Values: After Treatment</th>
<th>Control vs 1% Ethanol</th>
<th>Control vs 5% Ethanol</th>
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Kirwan 5
<table>
<thead>
<tr>
<th></th>
<th>After Treatment</th>
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</thead>
<tbody>
<tr>
<td>Change in Glucose</td>
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<td>0.17</td>
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<tr>
<td>Change in Lactate</td>
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<td>0.004 *</td>
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<tr>
<td></td>
<td>Control vs 1% Ethanol</td>
<td>Control vs 5% Ethanol</td>
<td></td>
</tr>
<tr>
<td>Change in Glucose</td>
<td>Inconclusive</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Change in Lactate</td>
<td>0.14</td>
<td>0.09</td>
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</tbody>
</table>
Table 2. P-Values for Treatment Comparisons below includes all p-values calculated from the t-tests that compared the pre-treatment to post-treatment measurements and post-treatment to post-exhaustion within each treatment group. The p-value labeled with an asterisk (*) symbol is statistically significant.

<table>
<thead>
<tr>
<th>T-Test P-Values: Control Glucose</th>
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</thead>
<tbody>
<tr>
<td>Pre-Exposure vs Post-Exposure</td>
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<tr>
<td>Post-Exposure vs Post-Exhaustion</td>
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</tbody>
</table>

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<thead>
<tr>
<th>T-Test P-Values: Control Lactate</th>
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<tbody>
<tr>
<td>Pre-Exposure vs Post-Exposure</td>
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<tr>
<td>Post-Exposure vs Post-Exhaustion</td>
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<table>
<thead>
<tr>
<th>T-Test P-Values: 1% Ethanol Exposure Glucose</th>
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<tbody>
<tr>
<td>Pre-Exposure vs Post-Exposure</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>Post-Exposure vs Post-Exhaustion</td>
<td>Inconclusive</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T-Test P-Values: 1% Ethanol Exposure Lactate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Exposure vs Post-Exposure</td>
<td>0.002 *</td>
</tr>
<tr>
<td>Post-Exposure vs Post-Exhaustion</td>
<td>0.06</td>
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<table>
<thead>
<tr>
<th>T-Test P-Values: 5% Ethanol Exposure Glucose</th>
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<tbody>
<tr>
<td>Pre-Exposure vs Post-Exposure</td>
<td>0.17</td>
</tr>
<tr>
<td>Post-Exposure vs Post-Exhaustion</td>
<td>0.17</td>
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<tr>
<th>T-Test P-Values: 5% Ethanol Exposure Lactate</th>
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<tbody>
<tr>
<td>Pre-Exposure vs Post-Exposure</td>
<td>0.0008 *</td>
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<tr>
<td>Post-Exposure vs Post-Exhaustion</td>
<td>0.05 *</td>
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</table>
**Figure 1. Mean Hemolymph Glucose for Treatment Groups** above shows the relationship between the three glucose measurements (mg/dL) for all three treatment groups. The calculated standard deviation is indicated by the error bars.

**Figure 2. Mean Hemolymph Lactate for Treatment Groups** shows the relationship between the three lactate measurements (mmol/L) for all three treatment groups. The calculated standard deviation is indicated by the error bars.
Discussion

The objective of this experiment was to investigate the effects of ethanol exposure on glucose, lactate and exercise ability. It was expected that glucose levels would increase following ethanol exposure, due to the stress response, and deplete following exercise, given it is the body’s main source of energy. It was expected that lactate would be at its lowest at pre-exposure and highest for post-exhaustion measurements, given its role in exercise. The data do not entirely support the hypothesis. For the control and 1% ethanol exposure groups, glucose did not increase as expected following treatment. It was expected that all levels would rise due to the introduction of a stressful environment (Caldari-Torres, 2018). However, crayfish in the 5% ethanol exposure group experienced changes expected with the hypothesis, given that its average glucose levels increased post-treatment, then decreased post-exhaustion. This result is consistent with the idea that stress can increase glucose levels, and it also follows that glucose levels decrease after exercise, given it is a source of energy (Nirupama, 2018).

Lactate levels continued to increase with each measurement, which is also consistent with the hypothesis. The greatest amount of increase in lactate occurred after exercise (Richter, 2013). The control group compared to the 1% and 5% ethanol treatment groups had a greater increase in lactate after exercise. This could be because ethanol, as an inhibitor of motor function and movement, had a greater effect on the crayfish ability to produce lactate (Valenzuela, 1997). The crayfish exposed to the 5% ethanol solution had much higher changes in lactate compared to the crayfish exposed to the 1% ethanol solution. However, overall, lactate levels were highest for all treatment groups following exercise to exhaustion. This could be accounted for by lactate’s responsibility in activity levels, as it increases during exercise (Richter, 2013).

There were numerous problems faced throughout this experiment that could have contributed to inaccurate results. Each crayfish, although randomly selected from the selection, differed in length and weight. This wavering of size could have influenced the way that each crayfish metabolized ethanol, glucose and lactate. Size can also have an effect on the crayfish’s level and amount of physical exertion and aggressiveness. Another variation for each crayfish was inconsistent timing of ethanol exposure. The
goal was to limit each crayfish’s ethanol exposure to two hours, however, there was variation as some test subjects were exposed for a longer or shorter period of time.

The measurements for glucose that recorded a level of 10 mg/mL read ‘Lo’ on the monitor. Following instructions from the glucose monitor, ‘Lo’ indicated levels below 20 mg/mL, therefore, it was advisable to label all ‘Lo’ glucose recordings as half of the lowest possible recording. The lowest possible recording for lactate was 0.8mmol/L and any measurement of lower value was read as ‘Lo.’ It was advisable for all ‘Lo’ measurements on the lactate monitor to be recorded as half its minimum value at 0.4mmol/L. While in the process of recording measurements, it was discovered that the glucose and lactate kits were expired. Given this, the results may have been skewed due to inaccurate readings. It is possible that measurements that were recorded as Lo, may have been of greater value.


