



SHORT-TERM EFFECTS IN THE AVERAGE HEART RATE OF

DAPHNIA MAGNA

UNDER DIRECT CAFFEINE EXPOSURE

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INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is the most widely used stimulant drug in the world (Yew, 2014). Caffeine consumption amongst humans is growing because caffeine is found in many consumables and pharmaceuticals that are ingested on a daily basis. According to a study by Nawrot *et al.* (2003), the average caffeine intake is 3 mg/kg in the United States. Caffeine is found in over 60 different plants like coffee beans, cocoa beans, and tea leaves. The amount of caffeine in products is dependent on how the consumable was prepared and the serving size. For example, boiled coffee may contain a maximum of 177mg/cup while an espresso may contain a maximum of 99 mg/cup (Nehlig, 1998). Medications, such as Midol and No Doz, contain 60 and 100 mg of caffeine per capsule, respectively (Yew, 2014).

Research shows that consuming less than 400 mg/day is not considered harmful for the body, (Nawrot, 2003), and positive effects of short term use become present. According to Glade (2010), the consumption of moderate amounts of caffeine may increase energy availability, decrease fatigue, enhance physical performance, increase alertness, quicken reactions, enhance short term memory, and improve reasoning. Taking in less than 400 mg/day of caffeine causes short term positive responses in the cardiovascular system. For example, the heart rate may decrease at levels as low as 75 mg (Glade, 2010). Caffeine acts as a vasodilator which widens the expanse of blood vessels and arteries, which increases blood flow and oxygen delivery throughout the body so the heart does not have to beat as fast to distribute blood throughout the body.

Although there are positive effects to consuming caffeine, there are negative responses if too much caffeine is consumed. The habitual consumption of more than 500-600 mg/day of caffeine may cause negative health effects from the long term use. Some negative effects may include an altered mental status, tremors, anxiety, poisoning, seizures, and cardiovascular issues (Glade, 2010). Long term cardiovascular responses may include a rise in heart rate and a widened pulse pressure. Other negative responses may be experienced, including the development of tachycardia (rise in heart rate or above normal heart beat), hypertension (high blood pressure), or arrhythmia (irregular heart beat) (Glade, 2010).

In order to illustrate the cardiovascular effects of caffeine, we hope to utilize bioassays, which are the use of organisms to test for chemical toxicity (“Bioassay: Background Information,” 2009). *Daphnia magna* (*D. magna*) are organisms frequently used in bioassay studies to test the impact substances may have on human beings due to their similarities. *D. magna* have myogenic heart tissue that is similar to mammals (Campbell, 2004). Not only are *D. magna* shown to behave similarly to humans, but they have many qualities that characterize them as good lab specimen. Internal structures are visible under a dissecting microscope due to the transparency of *D. magna*. Another beneficial aspect of using *D. magna* is that caffeine is not found in its natural environment; thus, *D. magna* have not built up a previous tolerance to the drug and can therefore indicate the true responses to caffeine. *D. magna* have been used to study humans multiple times in past comparative studies. In a study conducted by Campbell *et al.* in 2004, researchers tested the effects of lactose on *Daphnia pulex* and

determined *Daphnia pulex* behaved similarly to humans and are a dependable organism that could be used to test the effects of pharmaceutical substances on humans. The study introduced the idea that *Daphnia* respond to pharmacological agents that are attributed with affecting the ion channels and cell signals. When *Daphnia* are put into a medium they are absorbing and consuming the compound in the specific solutions they are exposed to. Baylor (1942) examined many pharmaceuticals on *D. magna*, such as acetylcholine, atropine, eserine, adrenalin, potassium, and nicotine, and found each treatment had diverse effects on the heart rate of *D. magna*.

The purpose of this study is to determine if *D. magna* heart rate behaves similarly to the heart rate of humans when exposed to low levels of caffeine. Since the amount of caffeine that is consumed by people each day is increasing, we are utilizing *D. magna* for this deterministic study, so we can quantify whether or not caffeine has an immediate effect of decreasing the heart rate.

In our experiment, we tested the impact of low levels of caffeine on *D. magna* heart rates. There were three caffeine treatments and a spring water control (0 mg of caffeine). The three caffeine treatments we chose contained 100, 200, and 300 mg of caffeine. We also used these treatments to evaluate the immediate short term effects on the average heart rate of the *D. magna*. Our null hypothesis is the average heart rate of *D. magna* in the caffeine treatment groups (100, 200, and 300 mg) will not be significantly different than the average heart rate of *D. magna* in the spring water control. The alternative hypothesis is the average heart rate of *D. magna* in the caffeine treatment groups (100, 200 and 300 mg) will be significantly lower than the average heart rate of *D. magna* in the spring water control.

METHODS

Our experiment was done in a biology laboratory at Frostburg State University in Frostburg, Maryland at the Compton Science Center from June 2015 to July 2015. Experimentation was done for four hours a day over the course of one week. The temperature in the laboratory varied from 15 to 21 degrees Celsius. Trials were completed with the room lights off, and only lower base dissecting microscope lights were on. When the *D. magna* are exposed to bright light, it can cause stress since they are sensitive to light contact (Carolina Biological Supply Company, 2006).

Three caffeine treatment solutions were created by diluting a 50 mg/mL caffeine stock solution made from spring water and lab grade caffeine powder. Three dilutions were made: 10, 20, and 30 mg/mL of caffeine. *D. magna* were exposed to 10 mL of each of these diluted solutions, resulting in 100, 200, and 300 mg of caffeine available to the *D. magna* in each exposure. Human heart rates are known to decrease from these treatments that present short term effects. Additionally, these ranges fall within the less than 400 mg that produce positive short term effects in humans. We used a control that consisted of spring water. The control group provided us with a value for the normal resting heart rate of *D. magna*.

For each treatment and the control, we used four petri dishes: one for the *D. magna* in spring water prior to testing, one for the caffeine treatment or the control, one for observation, and one for the recovery spring water

for *D. magna* after treatment. *D. magna* were transferred between each of the petri dishes using a pipet. One *D. magna* was moved at a time between the different dishes of solution. Fifteen *D. magna* were tested in each caffeine treatment with 60 *D. magna* being used in total. In the experiment, one *D. magna* was moved from the natural spring water to the caffeine treatment or the control for 10 minutes. After this exposure period, the *D. magna* were observed on the dissecting microscope for 30 seconds and multiplied by two to determine the heart beats per minute. Campbell *et al.* (2004) found that *D. magna* heart rate decreases or has an effect after five to 15 minutes. For our study, we examined the *D. magna* for 10 minutes since they seem to respond after this indicated time.

Following experimentation, we analyzed the heart rates of *D. magna*. We calculated the averages of each treatment group and the control group and compared the results. We calculated a 95% confidence levels for each of the averages, and T-tests were completed, with an alpha value of 0.05, to see if the treatment averages were significantly different from the control average.

RESULTS

Average heart rates for the control (204 beats per minute) were higher than all of the other treatments (100, 200, and 300 mg) with averages of 140, 103, and 84 beats per minute, respectively. The 95% confidence intervals did not overlap between the control and any of the three treatments. Our one tailed t-test showed that there was a significant difference between the average heart rate of the control and the average heart rates of the 100, 200, and 300 mg treatments ($p=7.0E-05$; $n=15$, $p= 3.6 E-09$; $n=15$, and $p=5.7 E-07$; $n=15$). Since the p-values were less than our alpha value of 0.05, we were able to conclude that *D. magna* do respond to these low doses of caffeine with an overall decline in heart rate.

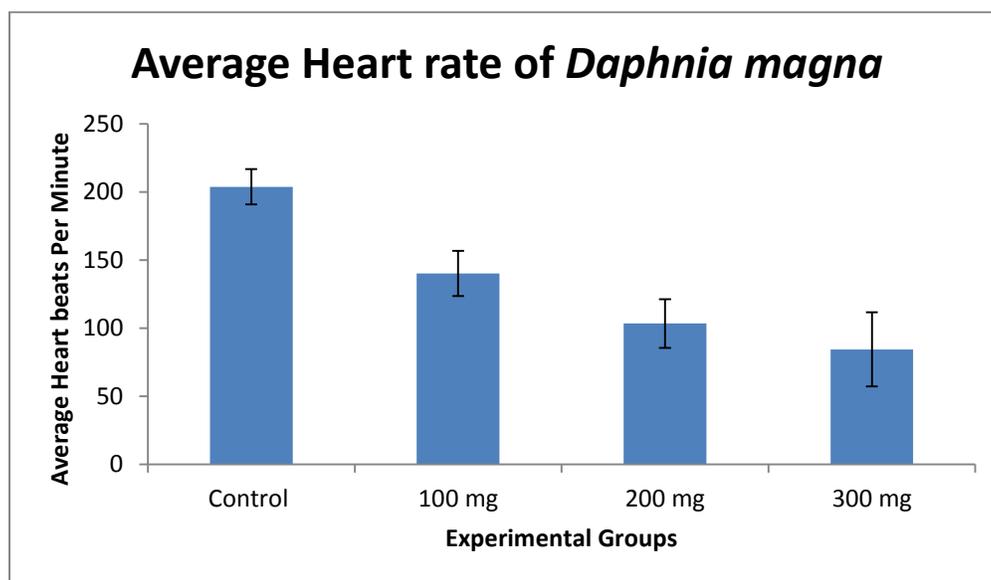


Fig. 1. – This figure shows a graphical representation of average heart rate of *Daphnia magna*.

CONCLUSIONS AND DISCUSSIONS

The main purpose of our experiment was to test if *D. magna* heart rate behaved similarly to humans at low levels of caffeine exposure. If *D. magna* behaved similarly to humans, their heart rates would be lower during the exposure to caffeine than during exposure to spring water. The 95% confidence intervals on the average heart rates do not overlap between treatments and the control (Figure 1) and all our p-values were less than 0.05 when running our t-tests, meaning that there was a statistically significant difference in heart rate when each caffeine treatment was compared to the control. This seems to suggest that in future studies involving caffeine *D. magna* may be valuable bioassay subjects. We rejected our tested null hypothesis which stated the average heart rate of *D. magna* in the caffeine treatment groups (100, 200, and 300 mg) will not be significantly different than the average heart rate of *D. magna* in the spring water control, and we accepted our alternative hypothesis which stated the average heart rate of *D. magna* in the caffeine treatment groups (100, 200, and 300 mg) will be significantly lower than the average heart rate of *D. magna* in the spring water control.

Our study had several limitations related specifically to our caffeine solutions. We only included 100, 200, and 300 mg exposures to caffeine and we did not test the full range of caffeine levels known to decrease human heart rates, therefore future studies should test wider ranges of caffeine exposures. Another problem arose when trying to make concentrated caffeine stock to use in dilutions. The caffeine started to crystallize at levels around 300 mg, making it fall out of solution. Future studies should research different forms or sources of caffeine as opposed to our lab grade caffeine powder or consider looking into different methods and recommendations for working with lab grade caffeine.

Further limitations arose from the experimental methods and design we used. During the experiment, we used low lighting to avoid distressing the *D. magna* (Carolina Biological Supply Company, 2006). Due to their sensitivity to heat, we were not able to use the overhead microscope lights because of the heat produced and as a result had trouble seeing the *D. magna*. We also had trouble seeing the *D. magna* under the dissecting microscopes with the room lights on and therefore decided to turn the room lights off. This made it easier to count the heart rate, but it was not optimal. In future studies, researchers may want to use a room with dim lights to make it easier to view and handle the *D. magna*.

Counting the fast heartbeats of *D. magna* was problematic. We found that our research groups developed different ways to record them. These methods included using tally marks, counting aloud, and keeping track mentally. For future studies, there should be a consistent counting method within the groups, such as using a cell counter or videotaping the heart beat and slowing the recording to obtain a more precise count. Furthermore, we had equipment limitations in our study. We used pipets to capture and transfer the *D. magna* while they were being moved between the four different petri dishes. The larger *D. magna* would get stuck in the pipets, which made the transfer process slow. Occasionally the *D. magna* were injured or killed in this process and we would have to redo the affected trial. Because of this process, there was the possibility that the *D. magna* would get

stressed out. Future studies should employ larger pipets or use a different means of transfer between petri dishes to make the process more efficient.

An error was introduced in our experimental design when the *D. magna* were exposed to the caffeine. Each treatment group only had one solution to which all of their 15 *D. magna* were exposed. Because of this, we cannot determine how much caffeine each *D. magna* was exposed to and what was still available to the *D. magna* that came afterwards. To resolve this issue in future studies, a different caffeine solution would have to be used for each individual *D. magna*, allowing researchers to say that the amounts the *D. magna* were exposed to were consistent.

Due to the limitations and errors within our study, many questions have arisen. We used various sizes and ages of *D. magna*, which could have affected our results if age or size impact caffeine uptake. One question that we would like to consider in future studies has to do with how sizes or ages of the *D. magna* affect their heart rates when exposed to caffeine. Our study only looked at how *D. magna* hearts behave at low exposures of caffeine, but in humans, after long-term exposure of high doses, different effects can be seen. We could also investigate if *D. magna* hearts behave the same as human hearts at higher concentrations of caffeine and longer exposures. If *D. magna* behave similarly to humans at other levels, they could prove to be useful in exploring more about how caffeine affects the human heart rate.

Some other questions also arose, such as how might the presence of other compounds combined with caffeine heighten or lessen the effects of caffeine on the heart rate. There is much concern in the realm of human health on how combinations of drug, or synergistic effects of drugs, impact individuals. In our study, *D. magna* were only exposed to lab grade caffeine solutions; however, there are numerous food and beverages with caffeine that could diminish or compound the effects on heart rates.

Our experimentation found *D. magna* to be suitable to use in bioassay studies of low doses of caffeine. Although they had similar responses to humans with decreasing heart rates, much is still unknown about caffeine and *D. magna*. Our experiment is just a starting point for future studies, not only examining caffeine but also determining if *D. magna* can be used to explore more compounds and their effects on human health.

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