The Effects of Stimulants on Invertebrates: Caffeine and Dietary Supplements

Caffeine and dietary supplements have been shown to play a role in the regulation and circulation of blood. If taken in excessive amounts, they may cause damage to the circulatory system or heart. To test the effects for myself, I administered both caffeine and dietary supplements on water organisms, specifically daphnia. The results for caffeine turned out as expected and further proved the effects of caffeine. For dietary supplements, the unexpected results showed that dietary supplements cause irregularity in heart rate. There was a very clear jump in daphnia heart rate between 30–60mins. for both stimulants. Sometimes, the heart rate would almost double when exposed to caffeine. With dietary supplements, there was dramatic inconsistency in the heart rate. The effect of dietary supplements on the daphnia’s heart rate was that it made the heart rate irregular and undependable. One might never know what might happen under the influence of dietary supplements.

INTRODUCTION

The Daphnia. The organism *Daphnia magna* was the specific organism used during these experiments. Daphnia are crustaceans whose main habitat is lakes, ponds, and quiet streams. They resemble fleas, hence their nickname, the “water flea.” Their main purpose is to harvest photosynthetic algae. Daphnia reproduce partly sexually, partly parthenogenically. Their average life span is 48 days.

Average Caffeine Intake for United States Consumers. The intake of caffeine among the average American has increased over the past several years, probably as our society has become more fast-paced and more stressful. ILSI North America sponsored research culminating in the Share of Intake Panel Survey, which tracks the average intake estimate for different substances across a particular group of people. Knight, *et al.* (2003) conducted the research on beverage caffeine intake among U.S. consumers, since, lately, increased concerns about the excessive amounts of caffeine consumed have been raised, especially regarding youth and pregnant women. High amounts of caffeine consumption have been proven through large amounts of research to adversely affect one’s health. The recommended amounts are as follows: for healthy adults, 400-450mg a day; for women who may be or are pregnant, 300mg a day; and for youth ages 4-5 years old, 45mg a day.

Knight, *et al.* (2003) attempted to discover whether the average
amount consumed had reached these levels yet through a targeted beverage survey, called the 1999 U.S. Share of Intake Panel. In this survey, caffeine intake from major dietary caffeine sources (coffee, tea, carbonated soft drinks) was measured in 10,712 beverage consumers. The results were safe, yet concerning. The mean caffeine intake in adult caffeinated beverage consumers was between 109mg and 170mg a day, while the 90th percentile was within 227mg and 382mg a day. For children between the ages of 1 and 5, the mean caffeine intake was 14mg a day, while children ages 6-9 years had an average intake of 22mg a day. The 90th percentile ranges were 37mg and 45mg a day, respectively. Pregnant women had an average consumption amount of 58mg a day (157mg a day at the 90th percentile), while women at the reproductive age, thus potentially pregnant or contemplating pregnancy, had 91-109mg a day of caffeine intake. At the 90th percentile, reproductive women were even at 229-247mg a day. Thus, although most people are within the healthy boundaries of caffeine intake, many, especially women and youth, should be more conscientious of their caffeine intake.

The Effects of Stimulants: Caffeine Effects on Recovery Sleep. High amounts of caffeine can have a negative effect upon health partly due to caffeine’s huge role in alertness, because sleep is often altered by high doses of caffeine in one’s system. A group of researchers at the Walter Reed Army Institute’s Research Department sought to explore the more detailed effects of caffeine, specifically recovery sleep. LaJambe, et al. (2005) conducted their survey in February 2005 and published it in Aviation, Space, and Environmental Medicine Journal.

Six habitually low caffeine users and three habitually high caffeine users were used in a randomized crossover design. (It is interesting to note that there was not an even number of high and low habitual users that participated in the survey. It is not explained why this is.) First, they were kept awake for 20 hours. Then, they were given doses of caffeine gum at 3 o’clock, 5 o’clock, and 7 o’clock.

There were three levels of caffeine administered: 0mg (used as a placebo), 100mg (the lower dose), and 300mg (the high dose). At 10 o’clock, three hours after the final dose, the subjects were allowed to sleep for eight hours. At this point, the subjects had gone 27 hours without any sleep whatsoever. 33 minutes after they were awakened, they took a PVT (Psychomotor Vigilance Test). They retook the test 65 minutes after awakening.

The results showed that the high dosage of caffeine led to reduced total sleep time and more restlessness. However, caffeine doses in those subjects with habitual caffeine use did not influence post-recovery sleep PVT performance. That the caffeine did not have as much of an effect on the habitual caffeine users as the other subjects means that most likely, people who habitually consume caffeine are not susceptible to lasting effects once they have slept. Overall, however,
caffeine was concluded to have exerted mild harmful effects on recovery sleep when following total sleep deprivation. These effects usually take place in the early sleep period and can be recovered from by sufficient sleep. This remedy of caffeine-affected sleep was shown by the lack of post-recovery sleep performance deficits. Habitual caffeine use was also affirmatively concluded to reduce the effects of caffeine, even if only minimally.

The Effects of Stimulants: Caffeine in Young Adults and Its Effects on Blood Pressure. In addition to the sleep issue, other concerns have been raised about caffeine’s effect, especially in youth, as the amount of youth that ingest caffeine regularly has grown. Generally, it has been established that caffeine increases awareness, energy, and activity, such as in research done by van Duinen, et al. (2005). These increases have been assumed to be the case because of increased blood pressure. As everything should be scientifically confirmed, however, several groups of scientists have worked on assays to conclusively show correlation between caffeine and increased blood pressure, especially in young adults. One of these groups was Savoca, et al. (2004), who originally published their findings in Archives of Pediatrics and Adolescent Medicine in 2004, republishing it in the American Journal of Hypertension in 2005.

Savoca, et al. (2005) properly understood the situation by realizing that no conclusive scientific evidence has been found to relate hypertension in adolescents with caffeine intake. In this new article, they took their findings one step farther by attempting to associate ambulatory blood pressure patterns and caffeine intake in youth, as well as reiterate their previous findings of specific comparisons at a single time point.

To bring the racial element in, they used eighty-two African Americans and non-Hispanic white adolescents, between the ages of 15 to 19 years old, as their subjects. For four days, they were put on a normal systolic BP food and beverage diet that was sodium-controlled. The amount of caffeine in these foods varied between three groups of these subjects. Over 24 hours (one day) of the four-day diet, all subjects’ ambulatory BP was measured and recorded. The factors that were being watched were ethnicity, caffeine, interaction of ethnicity and caffeine on BP, daytime versus nighttime hours, gender, and body mass index.

Their findings were not as pointed or drastic as I would have predicted. Actually, the level of dietary caffeine was positively associated with the daytime diastolic BP ($F=3.53$, $P=.03$, partial $R^2=0.07$) and daytime systolic BP ($F=3.1$, $P=.05$, partial $R^2=0.07$). Therefore, caffeine does in fact affect blood pressure. However, no correlation at all was found between caffeine intake and nighttime BP. That nighttime BP was not affected is contrary to what I would have assumed, due to the well-spread belief that if you have caffeine before bed, you’ll be kept up. This is generally true for most people, also.
The research question then remains whether or not caffeine alters activity and excitability because of a placebo effect or because of some other cause not BP related.

Savoca, et al. (2005) concluded through these new tests, however, that their previous findings were correct in deciding that caffeine consumption does impact the BP of adolescents, but especially during the daytime when sympathetic nervous system responses dominate BP control. Thus, caffeine affects the nervous system as well, as confirmed by previous articles and findings. However, Savoca, et al. (2005) also came to the same conclusion as I did in that more controlled studies that are more prevalent to today’s adolescents are needed. Otherwise, this data is useless to today’s world.

The Effect of Stimulants: Caffeine’s Effect on Mental Workload, Catecholamines, and Blood Pressure. In a more anatomical manner, however, how exactly does caffeine affect the human body? As commonly known, caffeine is a stimulant that affects the central nervous system, metabolism, and cardiovascular functions of the body. In 2003, research done by Karatzis, et al. (2005) was done to prove the adverse effects of caffeine on blood pressure. Furthermore, a study done by Papadelis, et al. (2003) conducted in February 2003 and published in Brain and Cognition, investigated caffeine’s specific effect upon catecholamines and their metabolites. A catecholamine is a type of amine used for signaling. Specifically, however, it helps support blood pressure. Some catecholamines include adrenaline, noradrenaline, and dopamine. Papadelis, et al. (2003) found that caffeine actually affects the excretion of catecholamines.

Papadelis, et al. (2003) found that after caffeine usage, the levels of urinary epinephrine and norepinephrine increased significantly. Epinephrine affects the cardiovascular system, generally speeding up the heart rate, as well as blood pressure. Norepinephrine is also known as noradrenaline, which has to do with the nervous system. Noradrenaline is the neurotransmitter of most of the nervous system. Thus, by affecting the levels of these two bodily chemicals, caffeine is changing the heart rate, blood pressure, and nervous system of the user. Papadelis, et al. (2003) discovered these effects of caffeine through a fairly simple, yet measured, assay. Each subject they used took a test testing cognitive performance, blood pressure, and catecholamine levels after oral administration of one cup of coffee and then three cups. Blood pressure was monitored before and after each stage. Catecholamines were collected and measured when at rest (as a control), after mental stress, after one dose of caffeine with stress, and after a triple dose of caffeine with stress. Papadelis, et al. (2003) was trying to make the connection between caffeine, stress, and catecholamine levels.

The results showed that there was an increase of adrenaline with one cup of coffee and a very significant increase of noradrenaline
(norepinephrine). This proves that caffeine does in fact affect adrenaline levels as well as the nervous system. Also, the assay showed that the triple dose of caffeine greatly increased the catecholamine levels, inferring that caffeine also affects and raises blood pressure. Mental workload increased the catecholamine levels, not surprisingly. Papadelis, et al. (2003) concluded, however, that this is a dose dependant effect of caffeine on catecholamines. Further scientific proof is needed to absolutely prove the connection caffeine has to blood pressure, catecholamines, and nervous system, but the research done by Papadelis, et al. (2003) adds to further proof.

Seizures in Connection with the Use of Dietary Supplements.
Caffeine is also an ingredient contained in most dietary supplements. Do dietary supplements have the same effect that caffeine does? Is the caffeine in dietary supplements more harmful than that taken through beverages? On the back of almost any dietary supplement is a warning that should be called a disclaimer. Almost every adverse health effect is covered, showing that for most dietary supplements, the potential effects are unknown and irregular. At the University of California, San Francisco, in 2005, Haller and colleagues decided to investigate reported incidents of seizures in persons using dietary supplements. At the time of Haller, et al.’s (2005) research, sixty-five cases of dietary supplement-associated seizures had been reported to the Food and Drug Administration’s (FDA) MedWatch system, between 1993 and 1999. Haller, et al. (2005) went through and evaluated each case based on possible causes, especially in relation to the use of dietary supplements, to determine which kinds of supplements tended to cause these effects, and to perhaps find some sort of pattern between the cases in order to determine potential risk factors for dietary supplement-related seizures.

As Haller, et al. (2005) was reviewing, they had mainly three reviewers looking at the cases, each one trying to find correlations and patterns. Their findings were as follows. Twenty seizures were probably related, thirteen possibly related, and ten completely unrelated to dietary supplement use. The results were altered because five cases were not really seizures at all, simply reported as such, and 17 cases did not have sufficient information from the report for Haller, et al. (2005) to deduce a determinant pattern or cause. Of these cases, weight loss and athletic performance enhancement were the two most cited reasons for taking the supplement. Weight loss categorized 45% of the users and athletic performance enhancement was the reason for 30% of the users. It was noted that most of the users were also using the supplement within the manufacturer’s guidelines. However, out of the twenty categorized as probably related, 19 were taking dietary supplements that contained ephedra, 14 with herbal caffeine, and one case did not have any herbal substances at all, but simply a combination of elemental salts.
Therefore, ephedra was looked at more closely since it was now seen as a very probable cause. Once ephedra’s potential connection was decided, Haller, et al. (2005) noticed that ephedra was contained within seven of the thirteen possibly related causes, and caffeine was also contained within five of these cases as well. Creatine, St. John’s wort, and ginkgo biloba were other dietary supplements that were seen in the possibly related seizure events. It can then be surmised that these five herbal ingredients could be potential causes of dietary supplement-related seizures.

In terms of other medical situations that might stimulate a negative effect of dietary supplements, three of the reported cases were associated with hypoglycemia, but there were also two cases with stroke and two with cardiac arrest. Thus, a correlation between the use of dietary supplements and effects upon the heart is seen in at least four reported cases.

With all this information, Haller, et al. (2005) concluded that ephedra was the most obvious connection with dietary supplement-related seizures. As such, ephedra was used in 27 of the 33 dietary supplement-related seizures reported over a seven year period. They further warn against the use of this herbal product, since it is seen to have negative health effects.

RESULTS

The Daphnia’s Heart Rate. In my first experimentation with daphnia, I made an attempt at measuring the daphnia’s average heart rate. I only got two trials with the same daphnia. The first count was 140 beats in 1min. and 4 secs. \( (140/64 = 2.19) \). The second count was 40 beats in 12 secs. \( (40/12 = 3.33) \). The next time, four more trials were made, with two different daphnia.

Daphnia #1:
- Trial 1 – 39 beats/13 secs. (3)
- Trial 2 – 21 beats/7 secs. (3)

Daphnia #2:
- Trial 1 – 50 beats/12 secs. (4.17)
- Trial 2 – 75 beats/17 secs. (4.41)

Average Heart rate: 3.65

The range of a daphnia’s heart rate is 2.19-4.41, with the average being 3.35.

Caffeine. Caffeine decidedly raises the heart rate of a daphnia depending on the dosage. In most high dosage situations, the daphnia used ended up dead. This may or may not be a direct factor of the amount of caffeine as opposed to other factors in the experiment. In those high dosage situations where the daphnia did not die, a very noticeable effect took place. In low dosage situations, sometimes an effect is seen, sometimes not.

High (5\( \mu \)g/mL) vs. Low (0.5\( \mu \)g/mL) Dosage. In the first experiment, in order to first test the effects of caffeine on the heart rate of a daphnia, a high dosage solution (5\( \mu \)g/mL) was made and a low dosage solution (0.5\( \mu \)g/mL) was made. Each solution was 10mL. Five daphnia were extracted from the tub using a dropper and placed in each petri dish. Both dishes were kept at room temperature. At 2hrs. and 23mins. of exposure, the five
Daphnia from the high dosage dish were taken out and their heart rates were measured. At 2hrs. and 36mins. of exposure, the five Daphnia from the medium dosage dish were taken out and their heart rates were measured. The caffeinated dishes were then disposed of and the Daphnia were stored in separate clean dishes with autoclaved water. The average heart rate for Daphnia on a high (5 µg/mL) dosage of caffeine was 2.45 beats/sec. The average heart rate for Daphnia in the low (0.5 µg/mL) dosage was 3.7 beats/sec. See Table #1 for individual heart rates.

Table 1. High Dosage vs. Low Dosage of Caffeine:
Daphnia were placed in both high and low dosages of caffeine, left for 2hrs. at room temperature, and then removed and their heart rates measured.

<table>
<thead>
<tr>
<th>HIGH DOSE (5 µg/mL)</th>
<th>Daphnia</th>
<th>Heart rate 1 (beats/secs.)</th>
<th>Heart rate 2 (beats/secs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dead</td>
<td>40/16 (2.5)</td>
<td>30/12 (2.5)</td>
</tr>
<tr>
<td>2</td>
<td>40/11   (3.64)</td>
<td>37/13 (2.85)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Dead</td>
<td>40/21 (1.9)</td>
<td>30/23 (1.3)</td>
</tr>
<tr>
<td>4</td>
<td>Dead</td>
<td>40/8 (5)</td>
<td>45/9 (5)</td>
</tr>
</tbody>
</table>

Table 2. Low Dosage (0.5 µg/mL) and Negative Control:
Daphnia were placed in a lower dosage of caffeine (0.5 µg/mL) for 1hr. at room temperature, and then removed and their heart rates measured.

<table>
<thead>
<tr>
<th>LOW DOSE (0.5 µg/mL)</th>
<th>Daphnia</th>
<th>Heart rate 1 (beats/secs.)</th>
<th>Heart rate 2 (beats/secs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dead</td>
<td>30/11 (2.73)</td>
<td>42/14 (3)</td>
</tr>
<tr>
<td>2</td>
<td>30/13   (2.31)</td>
<td>40/10 (4)</td>
<td>15/7 (2.14)</td>
</tr>
<tr>
<td>3</td>
<td>30/10   (3)</td>
<td>40/21 (1.9)</td>
<td></td>
</tr>
</tbody>
</table>

Low (0.5 µg/mL) Dosage #2 and Negative Control. In the next experiment, a solution of 0.5 µg/mL of caffeine was made in 10mL of autoclaved water, and placed in a sterile petri dish. 10mL of autoclaved water was placed in a separate sterile petri dish for a negative control. Five Daphnia were extracted from the tub using a dropper and placed in each petri dish. At 58mins. of exposure, the five Daphnia from the negative control dish were taken out and their heart rates measured. At 1hr. and 5mins. of exposure, the five Daphnia from the medium dosage caffeine assay were taken out and their heart rates measured. Once all were measured, they were placed in separate sterile petri dishes filled with tub water. The two solutions were disposed of. This assay was to make sure that the conditions were not affecting the Daphnia’s heart rate, since it was basically the same experiment as previous, except with a negative control also. It was found that the average heart rate of a Daphnia on a low (0.5 µg/mL) dosage of caffeine was 2.91 beats/sec. This is a great difference from the first assay, due perhaps to the particular Daphnia used or the conditions. The average heart rate for the Daphnia in the negative control was 2.45 beats/sec., which is also somewhat lower than...
previous experiments. See Table 2 for individual heart rates.

Medium (2.5µg/mL) Dosage vs. Low (0.5µg/mL) Dosage and Negative Control #2. In the past two assays, neither result has been very favorable in giving a distinct effect of caffeine. Thus, next, I gave a more medium dosage (2.5µg/mL) and the low dosage again, in order to compare. The medium dosage of 2.5µg/mL caffeine was made in 10mL of tub water. Two daphnia were extracted from the tub by means of a dropper and placed in the dish. They were taken out and their heart rates measured after 1hr. and 1min. To make the low dosage solution of caffeine, a 0.5µg/mL solution was made in 10mL of tub water. Two daphnia were placed in the dish and taken out and their heart rates measured after 1hr. and 4mins. For the negative control, 10mL of tub water was placed in a sterile petri dish, two daphnia were placed in it, and taken out and heart rate measured at 1hr. and 14mins. of exposure. Afterwards, all daphnia were placed in separate petri dishes filled with tub water. The solution dishes were disposed of. The average heart rate of the daphnia in the new medium dose was 8.78 beats/sec.; while the average heart rate of the daphnia in the low dosage was 3.75 beats/sec. These are more favorable, noticeable, and drastic results. The negative control average heart rate turned out as expected, also. The average heart rate for the daphnia within the negative control was 4.17 beats/sec. See Figure 1 for individual heart rates.

Timed Caffeine Assay. Daphnia from a new tub (shipment) were used from this point onward, since the old batch had run out. In the next experiment, I conducted a timed caffeine assay to see at what point the caffeine had its maximum effect. First, a solution of 2.5µg/mL was made in 10mL of tub water to make a caffeine dosage dish. For the negative control, 10mL of tub water was placed into a sterile petri dish. In each dish, six daphnia were placed. Two daphnia from the caffeinated dish were taken out after 30mins. and their heart rates measured. Two were taken from the negative control after 36mins. and their heart rates measured. This continued with two daphnia from the caffeinated dish after 45mins. and 56mins., and two daphnia from the negative control after 1hr. and 1hr. and 9mins. The daphnia used in this assay were then kept in two separate petri dishes filled with tub water. The solution dishes were disposed of. It appears the maximum time of effect of the daphnia heart rate takes place at 45mins. See Figure 2 for individual heart rates and results.
of caffeine, left at room temperature for the designated amounts of time, then taken out and their heart rates measured.

Timed Caffeine Assay (Medium Dose)

Dietary Supplements. Dietary supplements, as known by most people, affect the metabolism, heart rate, and blood pressure of the user. They are also known to sometimes have adverse effects upon the livelihood and health of the user. Since dietary supplements are stimulants, I decided to test their effects upon the daphnia as well as caffeine. I began going through basically the same trials that I did with caffeine on the daphnia with dietary supplements. Immediately, these effects were more drastic and noticeable. Later on, it appeared that the dietary supplement used, Hydroxycut, had similar effects to caffeine on the daphnia’s heart rate. I calculated a ratio of human weight to the prescribed amount of Hydroxycut with the weight of a daphnia, in order to make a solution that was proportional to a dosage of dietary supplement for a daphnia.

High (13.4975mg/mL) Dosage and Negative Control. First, a general assay of the dietary supplement’s effects on the heart rate of a daphnia was conducted, in order to see whether or not the dietary supplement would affect the daphnia. If so, I wanted to see what the effect would be. A solution of 13.4975mg/mL was made in 10mL of tub water. Five daphnia were placed into the dish, and taken out and had their heart rates measured after 1hr. and 4mins. For a negative control, 10mL of tub water was placed into a sterile petri dish with two daphnia placed in it. They were taken out and their heart rates measured after 1hr. and 9mins. All daphnia were then placed in separate petri dishes with tub water. The two solutions were disposed of. The average heart rate was 3.67 beats/sec., except that this was only the heart rate of one of the five daphnia used, since three of the five died and one’s heart rate was imperceptible. In the negative control run, the average heart rate was 4.56 beats/sec. See Table 3 for individual heart rates.

Table 3. High Dosage of Dietary Supplements and Negative Control: Daphnia were placed in a high dosage of dietary supplement and a negative control, left at room temperature for about 1hr., then taken out and their heart rates measured.

<table>
<thead>
<tr>
<th>Daphnia</th>
<th>Heart rate (beats/secs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11/3 (3.67)</td>
</tr>
<tr>
<td>2</td>
<td>N/D</td>
</tr>
<tr>
<td>3</td>
<td>Dead</td>
</tr>
<tr>
<td>4</td>
<td>Dead</td>
</tr>
<tr>
<td>5</td>
<td>Dead</td>
</tr>
</tbody>
</table>

Timed Dietary Supplement Assay. Just like in the caffeine assays, I needed to find when the maximum time of effect for dietary supplements
was. Thus, I ran a timed assay similar to the caffeine one. First, a dietary supplement solution of concentration 6.67mg/mL was made in 10mL of tub water in a sterile petri dish. For a negative control, 10mL of tub water was placed into another sterile petri dish. Six daphnia were placed in the supplemented dish. Six daphnia were also placed in the negative control. Two daphnia were taken out from each dish at about 35mins, about 50mins., and about 1hr. At each time interval, the four daphnia that were taken out had their heart rates measured. Afterwards, the daphnia were kept in two separate petri dishes filled with tub water. The two solution dishes were disposed of. It seems that the time at which the daphnia heart rate is most affected is about 50mins. just like the caffeine assay. In the negative control, the results turned out as expected, with no inhibiting conditions, and the average heart rate being 2.72 beats/sec. See Figure 3 for individual results.

Figure 3. Timed Dietary Supplement Assay and Negative Control: Daphnia were placed in a low dosage (6.67mg/mL) of dietary supplement, left at room temperature for the designated times, then taken out and their heart rates measured.

<table>
<thead>
<tr>
<th>Time Exposed (mins.)</th>
<th>Daphnia Heart Rate (beats/sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>3.67</td>
</tr>
<tr>
<td>36</td>
<td>4.5</td>
</tr>
<tr>
<td>50</td>
<td>3.7</td>
</tr>
<tr>
<td>50</td>
<td>2.6</td>
</tr>
<tr>
<td>60</td>
<td>2.8</td>
</tr>
<tr>
<td>60</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Medium (10.0005mg/mL) Dosage vs. Low (6.67mg/mL) Dosage and Negative Control #2. Since the first run of dietary supplements had most of the daphnia dead under whatever circumstances, I tried another run with two lower increments, a medium dosage (10.0005mg/mL) and a low dosage (6.67mg/mL). Both solutions were made in 10mL of tub water in two separate sterile petri dishes. For the negative control, 10mL of tub water was placed into a third sterile petri dish. Two daphnia were placed in each dish. At 45 mins. of exposure, the two high dosage daphnia were taken out and their heart rates measured. At 48 mins. of exposure, the two low dosage daphnia were taken out and their heart rates measured. Lastly, after 50 mins. the two negative control daphnia were taken out and their heart rates measured. The three dishes were disposed of. All daphnia were then stored in separate petri dishes filled with tub water. The average heart rate for the daphnia in the medium dosage was 3.75 beats/sec. and the average heart rate for the daphnia in the low dosage was 3.67 beats/sec. The negative control did not turn out as expected, interestingly enough, since the average heart rate was 4.84 beats/sec., a little higher than usual. See Table 4 for individual results and heart rates.

Table 4. Medium Dosage of Dietary Supplements, Low Dosage, and Negative Control: Daphnia were left in a medium or low dosage dish at room temperature for about 45mins. There was also a negative control ran. They were then taken out and their heart rates measured.

<table>
<thead>
<tr>
<th>MEDIUM (10.0005µg/mL) DOSE</th>
<th>Daphnia</th>
<th>Heart rate (beats/sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5/1 (5)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5/2 (2.5)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LOW (6.67µg/mL) DOSE</th>
<th>Daphnia</th>
<th>Heart rate (beats/sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24/6 (4)</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Caffeine has a very obvious effect upon the daphnia’s heart rate. In every experiment, the daphnia were observed to be very jumpy, excitable, and active after exposure to the caffeine. Their heart rates almost always increased, and in some situations doubled. This supports the present knowledge that states that caffeine increases heart rate. In fact, during the timed assay, one of the daphnia exposed for 45 mins. was extremely active and its heart rate was almost indiscernible due to its extremely fast pace.

At first, the daphnia that died in the assays we assume to have died due to the conditions. Originally, we were using autoclaved water in the petri dishes. Later, we used the treated tub water that the daphnia were stored in. There seemed to be fewer casualties once we switched to the tub water.

Regarding the unexpected high heart rates in the negative controls, we deduced that this was due to the different subjects used. Some of the daphnia were pregnant and thus may have had a faster than usual heart rate. Some were old while so were juveniles. All of these factors may have played a role in the heart rates.

Supporting the current claims by Savoca, et al. (2005), the juvenile daphnia were most affected by the caffeine. Usually, their heart rates were lower than the average daphnia’s. However, when exposed to caffeine, their heart rates rose at a higher proportionality than an older daphnia.

These results signify the potential danger of using excessive caffeine. The limitations of these experiments, however, were the use of invertebrates. We are unsure whether these results have direct connections to the human being, despite the correlation with current research on caffeine and humans. With the limited amount of time allotted, also, we were unable to reassess the heart rate under different conditions as well.

However, it is clear that keeping the heart at a faster pace for an extended period of time is not healthy and these assays do show that caffeine raises the heart rate. However, there is not much research on whether or not extended caffeine use impacts more adversely than higher dosages less frequently. Further research needs to be done on extended and habitual caffeine use. Once further research is conducted, caffeine and its effects will be better understood and thus better actions toward consumption will be known.

The dietary supplement had quite a different effect upon the daphnia heart rate. As we went through the experiments, there could never be any expectations, considering the continued inconsistency in resulting heart rates. At times, the daphnia’s heart rate would be lowered by exposure to the dietary supplement, while at other times, the heart rate would jump a significant amount. In terms of
activity, some of the daphnia used would be much more active and excitable, while some would be lethargic. These inconsistencies were at first considered effects of error. However, as more trials were run, with more favorable conditions, the same results appeared. This showed to us that the dietary supplements made the daphnia heart rate and activity unpredictable and irregular. The most poignant example of this is the medium vs. low dosage assay of dietary supplements. During this assay, both the lowest and highest daphnia heart rates calculated were the result of being exposed to the dietary supplement. We concluded that this is direct evidence of the dietary supplement’s adverse effect causing the heart rate to become irregular.

There were a lot of deaths within the dietary supplement assays. Upon closer evaluation, we found that the dietary supplement used, Hydroxycut, contained calcium. Daphnia cannot live in a habitat containing calcium, as found through research. Therefore, we attributed the deaths to be a potential effect upon the daphnia of the calcium environment.

Further research needs to be done about what correlations dietary supplements have with adverse health effects before any conclusive statements can be made. However, it is noticeable through past research and these assays that dietary supplements are risky and unstable. They may do what they intended to do but there may be severe health problems that result. The impact upon the heart alone, as seen through our research, is a dangerous enough effect of dietary supplements.

**MATERIALS AND METHODS**

**Maintaining the Daphnia Culture.** To maintain the daphnia culture, obtained from Carolina Biologicals, the daphnia were kept in a tub of treated tap water, and fed about 1/8th teaspoon of the provided food every Monday, Wednesday, and Friday.

**Measuring the Daphnia’s Heart Rate.** To measure the heart rate of a daphnia, one daphnia was extracted from the tub using a dropper, and placed on a slide. As much water as possible was taken up and disposed of, while one drop of Methyl Cellulose 1.5% was put on top of the daphnia. The Methyl Cellulose slows the daphnia down so that it is easier to observe the heart rate. The number of beats was counted while running the timer. The daphnia was then placed into a sterile petri dish with autoclaved water. This was repeated with three daphnia. Once these three were measured, the daphnia were placed back into the tub with the others.

**Making Solutions – Caffeine and Dietary Supplement.** A stock solution of caffeine was made of 5mg/mL in 10mL of water. Thus, 50mg of caffeine powder was needed (0.05g). 0.05g of caffeine powder was placed in a 15mL sterile tube and 10mL of autoclaved water was added. To mix, the tube was shaken until all the powder was dissolved. The dietary supplement used was Hydroxycut, which contains calcium, chromium,
potassium, hydroxagen plus (garcinia cambogia, gymnema sylvestra, glucomannam, alpha lipoic acid, willow bark extract, and L-carnitine), and hydroxy Tea (green tea leaf extract, caffeine, guarana extract). Each caplet contains 2.1613g of all these ingredients. A stock solution of 1g per 15mL (0.067g/mL or 67mg/mL) was made, as it was easiest to measure out 1g of the powder within the caplet. The caplet was cut open and the 1g of the powder was placed in a sterile 15mL tube. 15mL of autoclaved water was added. All was shaken to mix. Both solutions were stored at room temperature in their respective tubes.

**Assays.** For all the assays, the indicated number of daphnia was placed in 10mL of the appropriate solution in petri dishes and was left out at room temperature for the indicated time. They were then taken out, their heart rates measured by the above prescribed method, and placed into new sterile separate petri dishes filled with tub water.

**ACKNOWLEDGEMENTS**

I want to thank Dr. Julie Strong of Menlo School for all her advice in conducting the assays, ordering all the supplies, and giving me ideas.

**REFERENCES**


