Effects of Caffeine on Cardiac and Skeletal Muscle Stimulation: A Non-invasive Study Based on a Single Dose of Caffeine

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Abstract

According to previous research, caffeine is a known stimulant, which affects the nervous system through interactions with both A_1 and A_{2A} adenosine receptors as a competitive antagonist. The objective of this experiment was to analyze the physiological effects resulting from average daily caffeine consumption. We measured changes in reaction time, blood pressure, heart rate, and muscle activity after consuming a single dose of either a caffeinated or non-caffeinated beverage were measured. Females who consumed the caffeinated beverage showed a significant decrease in the QRS complex duration in the ECG compared to the control, indicating that the heart changed pace while consuming caffeine, no such difference was seen in males. For males, ECG amplitude, for the caffeinated group, was significantly increased compared to the control, possibly indicating increased heart contraction as a result of caffeine consumption, no difference was seen in the female groups. The average changes in blood pressure, as well as average value of a resting muscle(in mV) and peak-to-peak difference during a contraction, show that there is an overlap in the standard error ranges for either males or females. This indicates that there is no significant difference between caffeine and non-caffeine groups for these measured values.

Introduction

Caffeine is one of the most popular and well known stimulants used by man today. It is found in many different beverages including soda, coffee, and energy drinks as well as in other foods. This means that caffeine is used daily by millions of people which makes understanding its affects very important. Caffeine is known to affect many different physiological functions in humans including heart rate, body temperature, blood pressure, and locomotion.[2,3,4] Caffeine has also been know to have affects on cognitive abilities as well, including increased alertness and arousal, as well as decreased reaction time, better concentration and increased energy.[1,3] In terms of pharmacological effects, caffeine is very similar to other classical psychostimulants including

cocaine and amphetamine. One of the similarities of caffeine to these drugs is a reinforcing effect caused by the feelings of well being that these drugs can provide.[2] This is the reason caffeine, when consumed on a regular basis, can become very addicting. With increased use of caffeine around the world, understanding the addictive effects of caffeine has become an important topic of research. This research has lead to an understanding of the physiological mechanisms of caffeine's effects.

Caffeine is capable of interacting with both A_1 and A_{2A} adenosine receptors, which function as mediators of adenosine in the brain, located throughout the body including the forebrain and hypothalamus.[1] The effect caffeine has on muscle activity results from modulation of A_1 receptors located in the forebrain and A_{2A} receptors located in the hypothalamus.[1]   Caffeine's structural similarity to adenosine and adenine allows caffeine to act as a non-selective, competitive antagonist at both the A_1 and A_{2A} receptors. Through competitive binding and blocking of adenosine receptors in the brain, caffeine is capable of affecting the release of dopamine.[1] This is because A_1 and A_{2A} adenosine production plays a modulating role in dopaminergic neurotransmission due to the reciprocal antagonist allosteric interaction between the A_{2A} adenosine receptor and D_2 dopamine receptor hetromer. As a result, stimulation of adenosine receptors modulates dopamine receptors in an antagonistic matter.[1] Thus, caffeine is capable of counteracting the inhibitory affects of adenosine- activation of the adenosine receptors on dopamine receptors, which generating a significant increase in extracellular concentrations of dopamine. These interactions, which lead to the increased production of dopamine, results in the arousing affects of caffeine.[1]

In addition to the information about the mechanism of caffeine action on the body, considerable research has been done to determine the exact physiological effects of caffeine. Previous studies have already shown that caffeine can have a great impact on the cardiovascular system by causing increased heart rate and blood pressure which results in increased cardiac output.[3] Other studies indicate that increased caffeine consumption can cause decreased blood flow

to the cerebral cortex in non-habitual caffeine users. As well as an increase in oxygen consumption, and locomotor ability in different subjects.[2] Caffeine has also been shown to have varying affects on body temperature as well, depending upon the sex of the subject.[4] Cognitively, caffeine consumption causes increased arousal in subjects as well as noticeably decreased reaction times.[3] Thus, caffeine can have a wide range of physiological affects upon many different systems in the human body.

The focus of this experiment is to determine whether a single dose of caffeine (equivalent to one sixteen ounce soda) affects different physiological characteristics in humans. Based on this previous data as well as the mechanism of caffeine action, we believe that consumption of even a single dose of a caffeinated beverage will cause detectable changes in reaction time, cardiac output, and locomotor ability of test subjects. Specifically, we believe that consumption of caffeine will cause decreased reaction times, increased heart rates and blood pressures, as well as increased muscle activities in test subjects. In order to test this hypothesis, test one group will consume a standard caffeinated beverage and the other test group will consume the same beverage without caffeine. Reaction time, cardiac output, and locomotor ability will be tested before as well as after consumption to determine if there is a noticeable affect of caffeine on these physiological parameters.

Methods

To begin the experiment, each individual took baseline tests in order to establish their normal physiological parameters. Four different parameters were measured in this experiment reaction time, blood pressure, heart rate, and muscle activity. For the initial test of reaction time, subjects took a computerized automated reaction time test which tested their reaction time to a visual cue, the switching of the colors on a stoplight.[5] The average of the five trails was taken. To

determine blood pressure, a standard blood pressure cuff and stethoscope was used with blood pressure measured once after sitting for about five minutes.

To determine effects on heart rate and motor activity, Biopac software was used. An electrode was placed on each ankle and one on the right hand wrist. These electrodes were then connected to the computer. This allowed for the ECG of the individual to be measured and the measurement lasted for approximately one minute. Changes in heart rate were quantified by changes in the duration of the QRS complex as well as changes in the QRS complex ECG amplitude. For the initial test, baseline duration for the QRS complex and baseline ECG amplitude was taken after the subject had been sitting for at least five minutes. To use Biopac software to test this, an electrode was placed on each ankle and one on the right hand wrist. These electrodes were then connected to the computer. This allowed for the ECG of the individual to be measured and the measurement lasted for approximately one minute. From the resulting ECG, a standard section was chosen (defined as an area of relatively constant baseline and consistent QRS complex ECG amplitude). From this standard section, the QRS complex ECG amplitude and the QRS complex duration was measured from one of the peaks and taken as the baseline.

Changes in motor activity were measured as changes in baseline EMG amplitude as well as changes in peak to peak EMG amplitude caused by stimulation of the leg. An electrode was placed at the top of the leg (upper thigh) and two other electrodes were placed on the muscles just above the knee area. These were then connected to the computer to allow for EMG measurement using Biopac software. The stimulation used in this experiment was a simple reflex test in which the patellar tendon was struck with a reflex hammer. The resulting motor activity caused by the reflex test was the data of interest here. For the initial test, initial baseline EMG amplitude and initial peak to peak EMG amplitude caused by the reflex test was taken for each subject. The reflex test stimulation was done approximately five times for each subject (same intensity stimulation) and the

EMG peak with the largest amplitude was analyzed. From this peak, the peak to peak EMG amplitude was taken and from a flat baseline portion of the EMG, the baseline EMG amplitude was taken.

All four of the measurements above were taken prior to beverage treatment in order to get baseline readings. After these initial baseline measurements, each subject was unknowingly given one of two different beverages, either sixteen ounces of caffeinated coca cola or sixteen ounces of non-caffeinated coca cola. The caffeinated coca cola contained approximately 46 mg of caffeine, which is a typical amount for an average soda or cup of tea. After consumption of the soda, subjects were asked to wait fifteen minutes without any other activity before the tests were taken again. Fifteen minutes was a standard amount of time used in previous experiments in order to allow the caffeine to produce its full effects upon the subjects.[3] After the waiting period, each subject completed the four tests again. This data was then compared to the original baseline tests in order to determine any changes that may have been caused by the administration of the treatment.

Results

Systolic Blood Pressure

Systolic blood pressure measures the maximum pressure that circulating blood exerts on the walls of blood vessels. For males in the study that were given caffeine, the average systolic blood pressure (average of the measured systolic pressures for all males in group, n = 3) before beverage intake was found to be 119.67 ± 5.49 mmHg (error here and in further calculations is standard error of the mean, with three subjects each. All further average values represent the average of the measured values for all subjects in a particular group). After beverage intake, the average systolic blood pressure was found to be 125.00 ± 3.21 mmHg. The average change in systolic blood pressure (pressure after intake – pressure before intake, averaged over all subjects) (all further average changes were calculated in a similar matter for respective values) was found to be 5.33 ±

2.40 mmHg (Figure One). For non-caffeine males, the average systolic blood pressure before beverage intake was 129.33 ± 6.36 mmHg (n = 3) and after beverage intake was 137.00 ± 5.51 mmHg. The average change in systolic blood pressure was 7.67 ± 3.17 mmHg (Figure One). As seen in figure one, the standard error ranges overlap for the changes in systolic blood pressure for caffeine and non-caffeine males indicating no significant difference between the two treatments.

For females in the study that were given caffeine, the average systolic blood pressure before beverage intake was found to be 116.00 ± 5.85 mmHg (n = 3) and after beverage intake was found to be 117.00 ± 4.58 mmHg. The average change in systolic blood pressure was 1.00 ± 3.06 mmHg (Figure One). For non-caffeine females, the average systolic blood pressure before beverage intake was 126.00 ± 3.06 mmHg (n = 3) and after beverage intake was found to be 123.67 ± 8.41 mmHg. The average change in systolic blood pressure was -2.33 ± 6.17 mmHg (Figure One). As can be seen in figure one, the standard error ranges overlap for the changes in systolic blood pressure for caffeine and non-caffeine females indicating no significant difference between them.
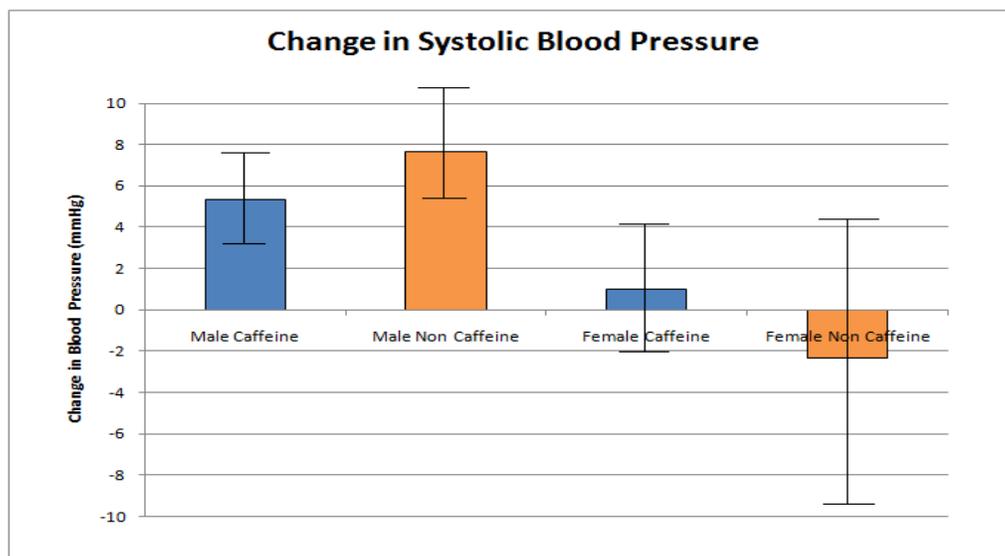

Figure One. Average change in systolic blood pressure for experimental subjects. Each value is based on an average of three replicates (three different subjects, n = 3). Error bars represent ± one standard error. Error bars overlap for caffeine and non-caffeine users in both male and female groups indicating no significant differences.

Diastolic Blood Pressure

Diastolic blood pressure measures the minimum pressure that circulating blood exerts on the walls of blood vessels. For the male caffeine group, average diastolic blood pressure before beverage intake was found to be 89.33 ± 4.06 mmHg (n = 3) and after beverage intake was found to be 90.00 ± 5.29 mmHg. The average change in diastolic blood pressure was found to be 0.67 ± 1.76 mmHg (Figure Two). For the non-caffeine male group, the average diastolic blood pressure before beverage intake was found to be 85.67 ± 3.38 mmHg (n = 3) and after beverage intake was found to be 89.00 ± 1.53 mmHg. The average change in diastolic blood pressure was found to be 3.33 ± 2.40 mmHg (Figure Two). As seen in figure two, the standard error ranges overlap for the average changes in systolic blood pressure for caffeine and non-caffeine males, which indicates no significant difference between them.

For the female caffeine group, the average diastolic blood pressure before beverage intake was found to be 84.00 ± 3.46 mmHg (n = 3) and after beverage intake was found to be 90.67 ± 0.67 mmHg. The average change in diastolic blood pressure was found to be 6.67 ± 2.91 mmHg (Figure Two). For the non-caffeine group, the average diastolic blood pressure before beverage intake was found to be 91.67 ± 8.17 mmHg (n = 3) and after beverage intake was found to be 93.67 ± 4.33 mmHg. The average change in diastolic blood pressure was found to be 2.00 ± 7.23 mmHg (Figure Two). As seen in figure two, the standard error ranges overlap for the average changes in diastolic blood pressure for caffeine and non-caffeine females, which indicates no significant difference between them.
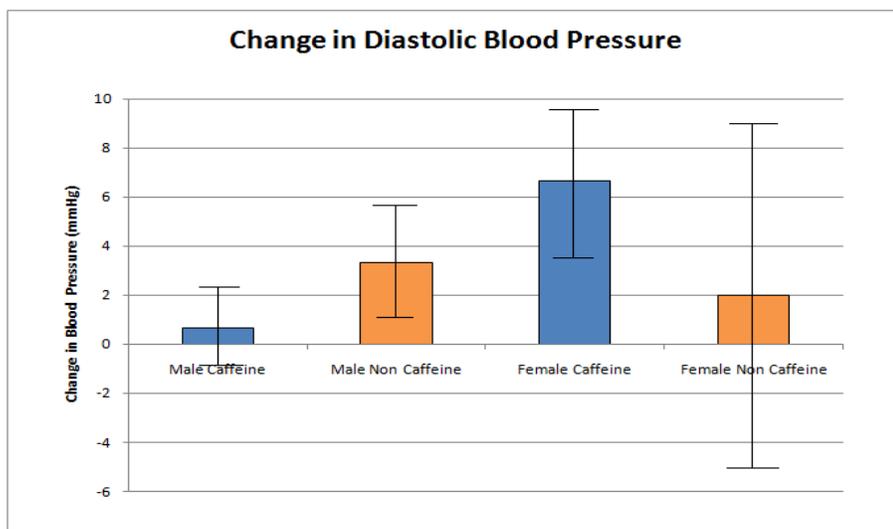
Figure Two. Average change in diastolic blood pressure for experimental subjects. Each value is based on an average of three replicates (three different individuals, n = 3). Error bars represent ± one standard error. Error bars overlap for caffeine and non-caffeine users in both male and female groups indicating no significant differences.

Reaction Time

A subject's reaction time measures how quickly a subject can respond to a stimulus. For the male caffeine group, the average reaction time before beverage intake was found to be 0.2983 ± 0.0425 seconds (n = 3) and after beverage intake was found to be 0.2630 ± 0.0327 seconds. The average change in reaction time was found to be -0.0354 ± 0.0144 seconds (Figure Three). For the male non-caffeine group, the average reaction time before beverage intake was found to be 0.2901 ± 0.0420 seconds (n = 3) and after beverage intake was found to be 0.2822 ± 0.0483 seconds. The average change in reaction time was found to be -0.0078 ± 0.0069 seconds (Figure Three). As seen in figure three, the standard error ranges do not overlap for the average reaction time changes for the caffeine and non-caffeine male groups, which indicates a significant difference between the two.

For the female caffeine group, the average reaction time before beverage intake was found to be 0.3067 ± 0.0366 seconds (n = 3) and after beverage intake was found to be 0.2787 ± 0.0278 seconds. The average change in reaction time was found to be -0.0280 ± 0.0135 seconds (Figure Three). For the female non-caffeine group, the average reaction time before beverage intake was found to be 0.2847 ± 0.0099 seconds (n = 3) and after beverage intake was found to be 0.2985 ± 0.0064 seconds. The average change in reaction time was found to be 0.0137 ± 0.0055 seconds

(Figure Three). As seen in figure three, the standard error ranges do not overlap for the average

reaction time changes for caffeine and non-caffeine female groups, which indicates a significant
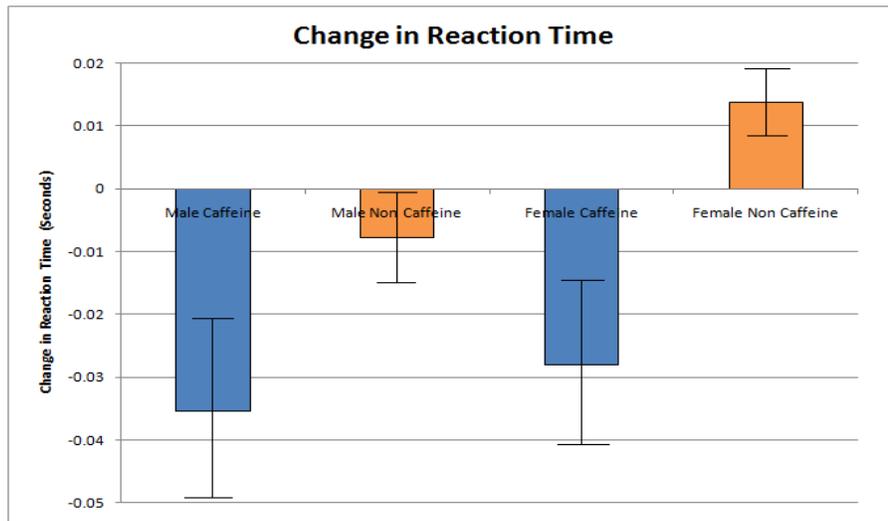
difference between the two.

Figure Three. Average change in reaction time for experimental subjects. Each value is based on an average of three replicates (three different individuals, n = 3). Error bars represent ± one standard error. Error bars do not overlap for caffeine and non-caffeine users in both male and female groups indicating significant differences between caffeine and non-caffeine groups.

ECG Amplitude

ECG amplitude is a measure of the depolarization that occurs within the heart during

contraction. Here it is believed that the greater the depolarization (thus greater ECG amplitude), the

greater the contraction resulting in increased cardiac output. For the male caffeine group, the

average ECG amplitude was 0.8948 ± 0.0560 mV (n = 3) before beverage intake and $1.2151 \pm$

0.0550 mV after beverage intake. This resulted in an average change in ECG amplitude of $0.3202 \pm$

0.0212 mV (Figure Four). For the non-caffeine male group, the average ECG amplitude before

beverage intake was 1.1016 ± 0.2454 mV (n = 3) and after beverage intake was found to be 1.2669

± 0.2253 mV. This resulted in an average change in ECG amplitude of 0.1653 ± 0.0440 mV

(Figure Four). As seen in figure four, the standard error ranges for the average changes in ECG

amplitude for the caffeine and non-caffeine groups do not overlap, which indicates a significant

difference between the two groups.

For the female caffeine group, the average ECG amplitude was 0.7855 ± 0.0936 mV (n = 3)

before beverage intake and after beverage intake was found to be 0.9001 ± 0.1905 mV. The

resulting average change in ECG amplitude was found to be 0.1146 ± 0.2140 mV (Figure Four). In

the non-caffeinated female group, the average ECG amplitude before beverage intake was found to

be 0.7191 ± 0.0701 mV (n = 3) and after beverage intake was found to be 0.7073 ± 0.0562 mV.

This resulted in an average change in ECG amplitude of -0.0118 ± 0.0559 mV (Figure Four). As

seen in figure four, the standard error ranges for the average changes in ECG amplitude for caffeine

and non-caffeine female groups overlap, which indicates no significant difference between them.
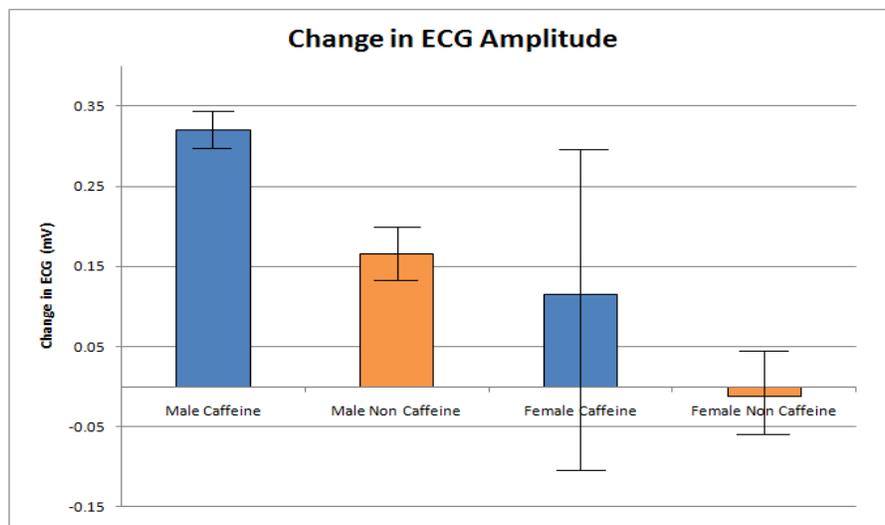

`**Figure Four. Average change in ECG amplitude for experimental subjects. Each value is
based on an average of three replicates (three different individuals, n = 3). Error bars
represent ± one standard error. Error bars do not overlap for caffeine and non-caffeine users
in the male groups indicating significant differences between caffeine and non-caffeine
groups. Error bars do overlap for caffeine and non-caffeine users in female groups indicating
no significant differences between them.**

ECG QRS Complex Duration

The QRS complex duration from an ECG trace represents approximately how long it takes

for the ventricles in the heart to become completely depolarized. Here this data is used to represent

the length of a heartbeat since a long duration would represent slower depolarization and thus a slower heart beat duration and vice versa. For the caffeine male group, the average QRS complex duration before beverage intake was found to be 0.0597 ± 0.0023 seconds (n = 3) and after beverage intake was found to be 0.0597 ± 0.0037 seconds. This resulted in an average change in QRS complex duration of 0.0000 ± 0.0021 seconds (Figure Five). For the non-caffeine male group, the average QRS duration was found to be 0.0813 ± 0.0015 seconds (n = 3) and after beverage intake was found to be 0.0810 ± 0.0085 seconds. This resulted in an average change in QRS complex duration of -0.0003 ± 0.0084 seconds (Figure Five). As can be seen in figure five, the standard error ranges for the average changes in QRS complex duration for caffeine and non-caffeine male groups overlap, which indicates no significant difference between them.

For the female caffeine intake group, the average QRS complex duration before intake was found to be 0.0650 ± 0.0026 seconds (n = 3) and after beverage intake was found to be 0.0617 ± 0.0030 seconds. This resulted in an average change in QRS complex duration of -0.0033 ± 0.0003 seconds (Figure Five) for the caffeine group. For the non-caffeine intake group, the average QRS complex duration before intake was found to be 0.0620 ± 0.0020 seconds (n = 3) and after beverage intake was found to be 0.0790 ± 0.0032 seconds. This resulted in an average change in the QRS complex duration of 0.0170 ± 0.0051 seconds (Figure Five) for the non-caffeine group. As can be seen in figure five, the standard error ranges for the average changes in QRS complex duration for caffeine and non-caffeine females do not overlap, which indicates a significant difference between them.
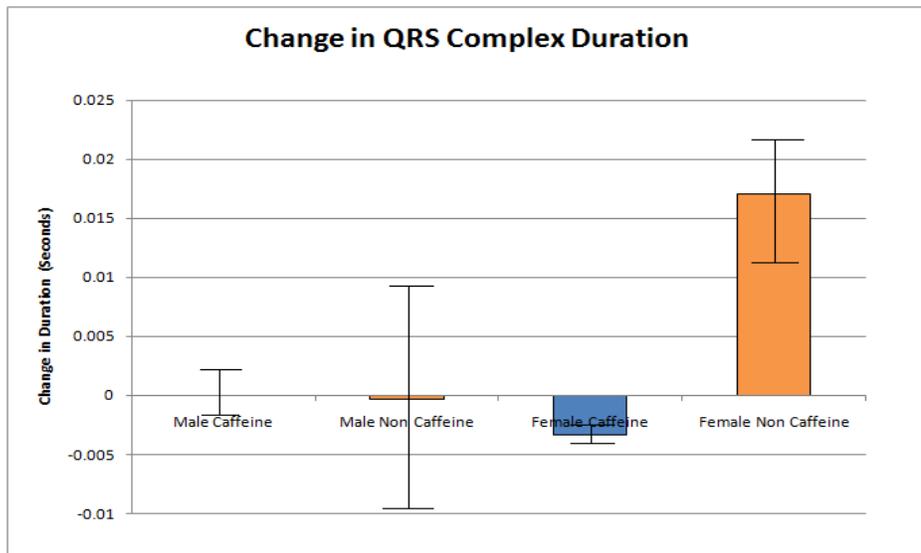
Figure Five. Average change in QRS complex duration for experimental subjects. Each value is based on an average of three replicates (three different individuals, n = 3). Error bars represent ± one standard error. Error bars do not overlap for caffeine and non-caffeine users in the female groups indicating significant differences between caffeine and non-caffeine groups. Error bars do overlap for caffeine and non-caffeine users in male groups indicating no significant differences between them.

EMG Mean Baseline

The EMG mean baseline is a measurement of the baseline level of motor activity that can be measured for a particular subject. EMG measures the electrical activity of an individual which can then be related to baseline levels of motor activity. For the male caffeine intake group, the mean EMG baseline before beverage intake was found to be -0.0105 ± 0.0065 mV (n = 3) and after beverage intake was found to be -0.0046 ± 0.0001 mV. The average change in EMG mean baseline that resulted from this was found to be 0.0059 ± 0.0064 mV (Figure Six). For the non-caffeine male group, the mean EMG baseline before beverage intake was found to be -0.0046 ± 0.0002 mV (n = 3) and following beverage intake was found to be -0.0073 ± 0.0028 mV. The resulting average change in EMG mean baseline was -0.0027 ± 0.0028 mV (Figure Six). As can be seen in figure six, the standard error ranges for the average changes in EMG mean baseline for caffeine and non-caffeine groups overlap, which indicates no significant difference between them.

For the female caffeine group, the average EMG mean baseline before beverage intake was found to be -0.0046 ± 0.0002 mV (n = 3) and after beverage intake was found to be -0.0046 ± 0.0001 mV. This resulted in an average change in EMG baseline of 0.0001 ± 0.0002 mV (Figure Six). For the non-caffeine female group, average EMG baseline before beverage intake was found to be -0.0046 ± 0.0001 mV (n = 3) and after beverage intake was found to be -0.0046 ± 0.0001 mV. The resulting average change in EMG baseline was found to be -0.0001 ± 0.0001 mV (Figure Six). As can be seen in figure six, the standard error ranges for the average changes in EMG mean baseline for caffeine and non-caffeine females overlap, which indicates no significant difference between them.
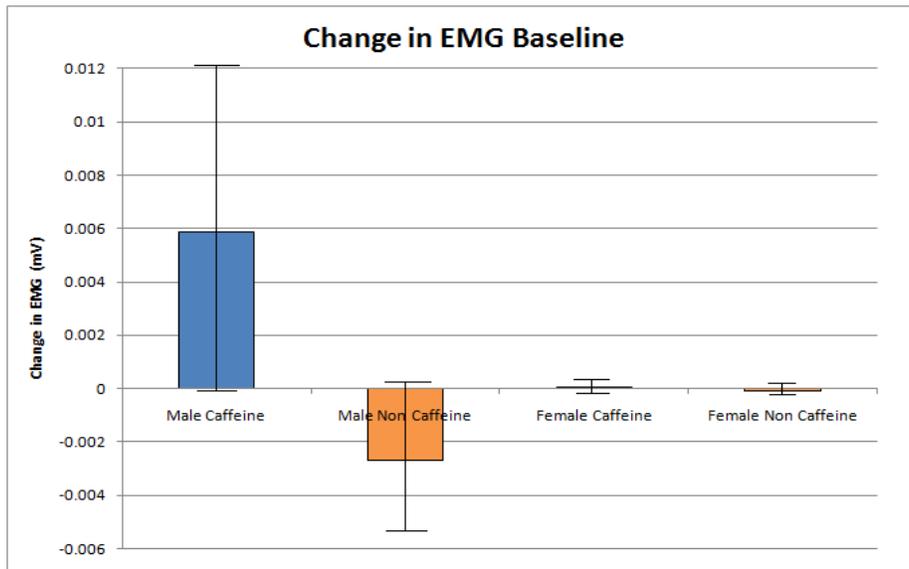

Figure Six. Average change in mean EMG baseline for experimental subjects. Each value is based on an average of three replicates (three different individuals, n = 3). Error bars represent ± one standard error. Error bars overlap for caffeine and non-caffeine users in both male and female groups indicating no significant differences.

EMG Integrated Peak to Peak

The EMG integrated peak to peak is a measurement of the motor activity that in this case resulted from a simple reflex test of a particular subject. This measurement measures the electrical activity of the individual that resulted from a reflex test which is then relatable to the motor activity that has been stimulated. For the male caffeine group, the average peak to peak integrated value

before beverage intake was found to be 1.2133 ± 0.4330 mV (n = 3) and after beverage intake was found to be 1.2896 ± 0.4936 mV. The average change in peak to peak integrated value was 0.0762 ± 0.0980 (Figure Seven). For the non-caffeine male group, the average peak to peak integrated value before beverage intake was found to be 1.4162 ± 0.3936 mV (n = 3) and after beverage intake was found to be 1.1621 ± 0.2972 mV. The average change in peak to peak integrated value was - 0.2541 ± 0.6881 mV (Figure Seven). As can be seen in figure seven, the standard error ranges for the average changes in integrated peak to peak values for caffeine and non-caffeine males overlap, which indicates no significant difference between them.

For the female caffeine group, the average integrated peak to peak value before beverage intake was found to be 0.6673 ± 0.2024 mV (n = 3) and after beverage intake was found to be 1.0206 ± 0.5072 mV. The average change in peak to peak integrated value was found to be 0.3533 ± 0.3348 mV (Figure Seven). For the female non-caffeine group, the average integrated peak to peak value before beverage intake was found to be 1.0522 ± 0.2071 mV (n = 3) and after beverage intake was found to be 1.1275 ± 0.2928 mV. The average change in peak to peak integrated value was found to be 0.0753 ± 0.1038 mV (Figure Seven). As can be seen in figure seven, the standard error ranges for the average changes in integrated peak to peak values for caffeine and non-caffeine females overlap, which indicates no significant difference between them.
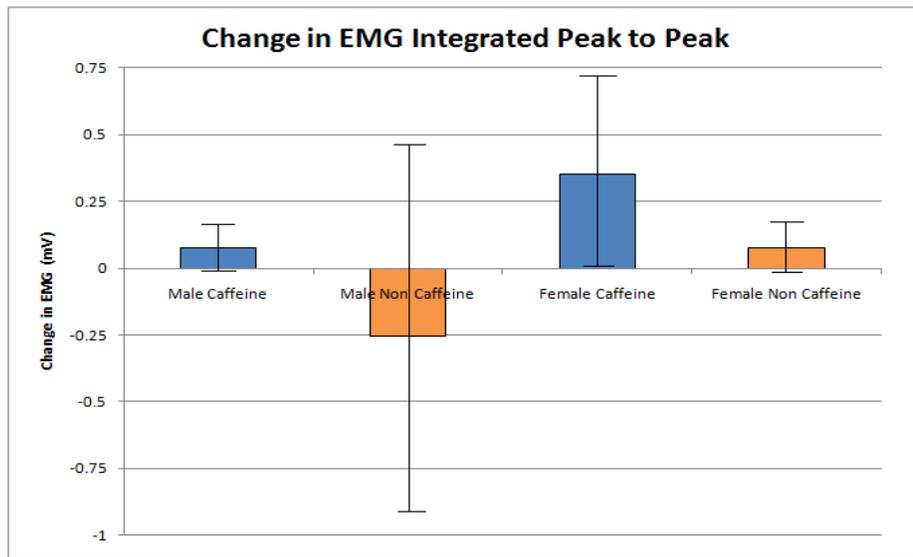
Figure Seven. Average change in EMG integrated peak to peak for experimental subjects. Each value is based on an average of three replicates (three different individuals, n = 3). Error bars represent ± one standard error. Error bars overlap for caffeine and non-caffeine users in both male and female groups indicating no significant differences.

Discussion

To compare the non-caffeine group and the caffeine group for males and females, we subtracted the observed value before beverage intake from the observed value after beverage intake for each individual. We then averaged these changes to collect average values for changes in blood pressure, reaction time, ECG amplitude, and EMG integrated peak-to-peak. To determine if there were significant differences between non-caffeine and caffeine groups in males and females, standard error ranges for measured changes in each value were calculated. Standard error ranges which overlapped between caffeine and non- caffeine groups indicated data which was not significant enough to reject the null hypothesis of no difference following caffeine consumption. A lack of overlap would indicate a significant difference in the measured value.

For females, there was a significant difference in the QRS complex duration in the ECG measurements, indicating that the heart changed pace following caffeine consumption. The reaction time test also showed significant differences for females, as was expected. For males, the ECG amplitude was significantly different from non-caffeinated groups to the caffeinated groups. This

could imply increased heart contraction among males as a result of caffeine consumption. The reaction time for males also showed a significant difference between the two groups, which was also expected.

As previously stated, caffeine brings about physiological changes through its role as a noncompetitive antagonist to A_1 and A_{2A} receptors. Through their interactions, there is an increase in extracellular concentrations of dopamine, which leads to the stimulating affects of caffeine. The implications of dopamine production are capable of explaining the decrease in reaction time for males and females, decrease in QRS complex for females, and a decrease in male ECG amplitude following caffeine consumption. These results show that female may not have as many A_1 or A_2 adenosine receptors compared to males. For the systolic blood pressure measurements, male systolic blood pressure appears to increase regardless of what beverage was consumed. These results imply that males may be more susceptible to the power of suggestion.

However, the average changes in blood pressure, as well as baseline and integrated EMG, show that there is an overlap in the standard error ranges for both males and females. This indicates that there is no significant difference between caffeine and non-caffeine groups for these measured values. There were also no significant differences in QRS complex duration among male subjects and for female subjects there were no significant differences in ECG amplitude. However, based on previous studies' strong evidence in showing that caffeine intake causes higher blood pressure, it was concluded that the limitation of participants in the study likely affect the ability to analyze and conclude causation effects in the data set. The lack of data in the study reflects upon the overlap of standard deviations among males and females with and without caffeine consumption. Regardless of the effects of caffeine consumption, other factors that may have contributed to the trend in observations were considered. Such factors may include the participant's genetic disposition or physiological health problems. However, since the study was conducted using four

measurements, there is a greater probability that these results will directly correlate with the results. It is difficult to isolate one predominant factor in this study since many variables may play a contributing role in elevating blood pressure and increasing heart rate. Each group member had conducted and researched how to collect the four measurements, thus the standard error in variation between measurements was minimized. Thus, there were no problems with equipment usage. The reaction time changed among caffeine and non-caffeine subjects, which was expected. This means that the reaction time data was relevant data that showed a trend that was expected. This indicates that the lack of differences in the other tests was due to lack of sample size instead of mechanical error. The participants in the study remained in a separate area from the data collection and caffeine preparation in order to reduce the chance of obtaining results due to the placebo effect. One problem we faced is the amount of time the experiment requires. We did not anticipate that the experiment would last as long as it did. We have solved this problem by staggering the testing of our subjects to eliminate their waiting time after beverage consumption. Our study does not take into consideration or record the participants' previous consumption of caffeine in the past 24 hours. This lack of completeness may skew the data due to prior exposure of the stimulant. We were not able to account for test subjects that may have been habitual caffeine consumers. It is possible that they already have a tolerance built up, which may have negatively affected the results in our experiment. We attempted to prevent this problem by ensuring that subjects did not have caffeine two hours before this experiment. However, this would not eliminate the problems raised due to the habitual caffeine users. Future scientists may cope with limitations in the study by collecting preliminary data based on the participant's heath history and activity in the past 24-48 hours. A larger, more randomized sample size would also lead to greater statistical power, decreased variability, and reliably indicate that the observed significant changes in reaction time are caused by caffeine consumption.

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