The role of CYP1A2 and ADORA2A in Individual Response to Caffeine Consumption under Anaerobic Conditions

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ABSTRACT

Caffeine is the most commonly used psychoactive drug in the world, showing ergogenic effects and links to myocardial infarction, hypertension, prediabetes, and neurodegenerative diseases. As such it has wide implications in medicine, athletics, and public health. Studies have shown that the reception and metabolism of the compound varies significantly among individuals, and consequently interindividual response to its consumption. The pharmacokinetics of the drug are almost exclusively dictated by the cytochrome p450 enzyme CYP1A2 while the adenosine neuroreceptor ADORA2A heavily influences the drug's pharmacodynamics. Polymorphisms of the -163 A>C CYP1A2 and the 1976 T>C ADORA2A are thought to influence these interindividual responses **PURPOSE:** To further understand the mechanism of interindividual drug response, this project focuses on the effect of caffeine consumption on anaerobic exercise. **METHODS:** 11 female college athletes completed two maximal 30 second Wingate anaerobic bike tests (WAnT30) on a Velotron cycle ergometer, once after ingesting a caffeine (5mg•kg-1 BW) and another after a placebo pill (maltodextrin), in a double-blind fashion. The trials were separated by 2-7 days, and the pills were administered 60 minutes before testing. A mouth rinse of 0.9% NaCl was used to obtain buccal epithelial cells, DNA was extracted using QiAmp Mini spin columns, and cells were lysed with proteinase K. Peak power ($W•kg^{-1}$), anaerobic capacity ($W•kg^{-1}$), and total power output ($W•kg^{-1}$), were recorded during each test by Velotron software, and analyzed by comparing the metrics of each trial to see how caffeine affected that athlete's ability to complete the test. Subjects' genotypes and metabolic phenotypes will be determined by running the extracted DNA samples through PCR, and ELISA. Allelic discrimination will be obtained using TaqMan® SNP Assay for CYP1A2 (rs762551) and ADORA2A (rs5751876) and a One-Step qPCR. Each sample will be run in duplicate positive and negative quality controls. Each variable will be analyzed using a factorial ANOVA with repeated measures ($p > 0.05$). ANOVA tests will be run to determine the main effect of caffeine as well as any significant effect of the enzyme or neuroreceptor.
RESULTS: There was no significant difference between placebo and caffeine tests in regard to anaerobic capacity, anaerobic power, total power, or percent decline

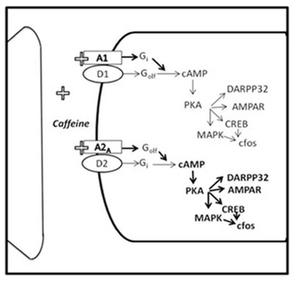


Figure 1

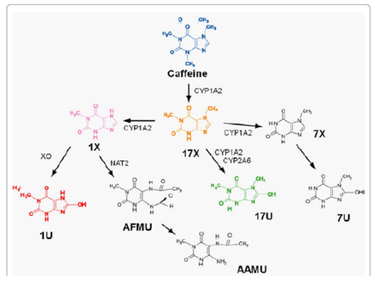


Figure 2

INTRODUCTION

The rs762551 single nucleotide polymorphism (SNP) in the CYP1A2 genotype is strongly correlated to the speed of an individual's caffeine metabolism, which can vary up to 40-fold between and within individuals. Rs762551 has two alleles, the wild-type A, or the variant type C. Variant alleles encode a CYP450 enzyme that has reduced or no activity, so genotypes AC or CC are considered "slow metabolizers" while the AA genotype are considered "fast metabolizers."[2,3,4] Studies show the interindividual variation of the CYP1A2 enzyme SNPs greatly affects aerobic athletic performance in the presence of caffeine. The endurance of fast metabolizers is greatly improved after consumption of caffeine, while heterozygous slow metabolizers see no effect, and homozygous slow metabolizers experience hindrance of their performance, by the greatest margin of change.[2] Genotyping CYP1A2 has shown a strong correlation to caffeine response; however, the role of genetic polymorphisms in metabolism is still being investigated. Therefore, phenotyping is necessary to minimize the effect of confounding influential factors. The ADORA2A adenosine receptor may also have a role in reinforcing these responses. . The A_{2A} type receptors are responsible for caffeine's increase of arousal, and the rs5751876 SNP is responsible for the level of arousal. Individuals with the TT genotype are deemed insensitive to caffeine, while those with the CC genotype are classified as being "sensitive."[4.] The relationship, however, between caffeine consumption and anaerobic exercise is still unclear. Anaerobic experiments have been individually inconclusive or contradictory with other results. By designing an anerobic test to be short and supramaximal, the subjects must rely on their glycolytic enzymes and speed of glycolysis reactions to provide their energy and power, as the demands of the test exceed the rate of oxygen utilization in the respiratory chain reactions, or aerobic respiration. The most widely used anaerobic test is the Wingate Anaerobic Test, which measures anaerobic power, mean anaerobic power, total work, and fatigue peak. Previous experimental results preclude the necessity for Wingate tests longer than 30 seconds as maximum power is not affected by an increase in time[1].

PURPOSE

To examine the effects of caffeine, CYP1A2 (rs762551), ADORA2A (rs5751876) genetic variants on anaerobic performance.

METHODS

Wingate Protocol
The participating subjects were female athletes from Messiah University, to limit gender and other demographic variables. The tests were be preceded by a familiarization trial during which height, body weight, and cycling position on a cycle ergometer will be recorded. A modified WAnT30 test of only 5% bodyweight resistance was performed. Between the familiarization and the first trial, each subject kept a 3-day record of their habitual caffeine ingestion. An hour before completing each 30 second test, the subject was be given a pill in a double-blind fashion; either a maltodextrin placebo or one containing an individualized amount of caffeine (5mg•kg-1) formulated in reference to bodyweight at familiarization. The placebo was maltodextrin. Before the test, the athlete moved through a five-minute warm up routine; including five five-second sprints. The test was an all-out 30-second cycle on a Velotron (SRAM, Chicago, IL) bicycle with 7.5% bodyweight resistance. The WAnT 30 protocol calculated peak power, anaerobic capacity, and total power output every 0.1 seconds. A 3-4 minute cooldown for recovery followed each test.

ADORA2A and CYP1A2 Protocol
A buccal epithelial cell sample was be collected from each subject at the familiarization trial by means of a 0.9% NaCl saline mouth rinse that the athletes swished in their mouth for 15-20 seconds. DNA isolation was accomplished using the Qiagen DNA isolation kit (BioVision, Milpitas, CA). The cell samples were combined with proteinase k to lyse the cells, for access to the DNA. A TaqMan® Genotyping Master Mix, containing the TaqMan® SNP Assay, will be added to the DNA and samples plated. The DNA solution will be amplified using qPCR. From this reaction, an allelic discrimination plot will be generated, visually representing allelic frequency. From this plot, the genotype (TT, TC, or CC) of each individual will be determined.

ELISA Protocol
1:400 dilutions of the saliva samples will be made in fresh tubes with EIA buffer (phosphate buffered saline with bovine serum and a preservative), starting with 2 µL of each saliva. 20 µL of each will be added to the ELISA plate, highest to lowest, down each column. A EIA buffer control will also be run. The time-course samples will be loaded down the column, t=0 to t=60 min. Caffeine-HRP will be addedto each well and mixed. After room temperature incubation for 45 minutes, remaining liquids will be drained, wells washed and mixed (x4) K-Blue Substrate will be added to each well , and the plate will be incubated at room temperature for 30 minutes. Absorbance will be measured at 650 nm, and the caffeine concentration will be plotted vs the normalized absorbance. The standard curve will be used to calculate the concentration of caffeine in each time-course sample. It is expected that the curve will follow first-order elimination kinetics.

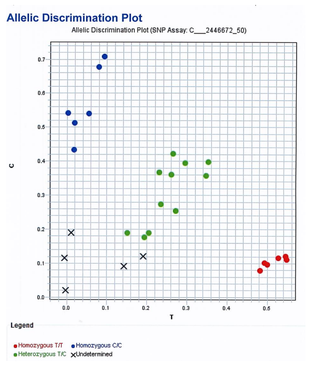
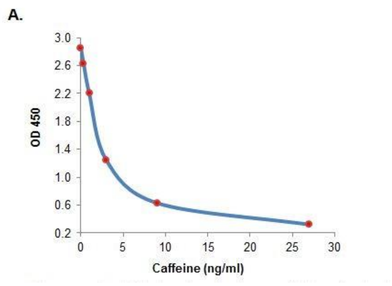


Figure 3



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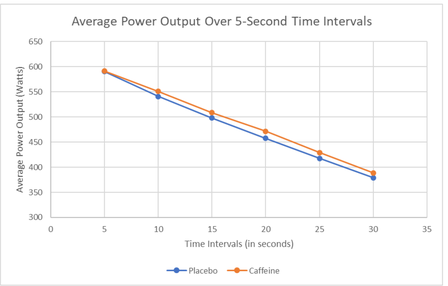


Figure 4

T-test for Caffeine vs. Placebo	
Metric	P-value
Anaerobic Power	0.27417639
Anaerobic Capacity	0.32751582
Total Power	0.20027873
Percent Decline	0.24902728

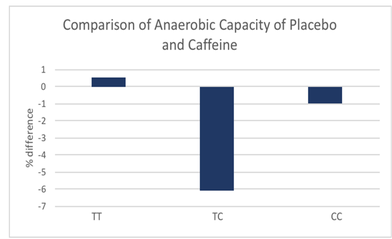
Table 1.

Results

The data suggests that there is no significant difference in power output between placebo and caffeine trials over the six 5-second intervals (Figure 4) . Single tailed t-tests were run for several variables (anaerobic capacity, anaerobic power, total power, and percent decline), but showed no significant difference between trials for any variable (Figure 5).

Conclusion

The results indicate that there is no significant effect of caffeine on the subjects' test performance as whole. However, research has shown that the genotype of the CYP1A2 and ADORA2A genes have important role in an individual's metabolism and reaction to caffeine. Past studies done at Messiah University have shown a decrease in performance of subjects with the TC/CC ADORA2A gene and XX CYP1A2 gene (Figures 5 and 6). This would explain why, as a whole, no improvement was seen in the metrics reported. As this study progresses, and subgroups of genotypes and phenotypes can be made, it is hypothesized that genotypes will correlate to significant difference in performances.



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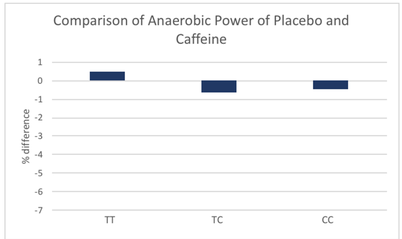

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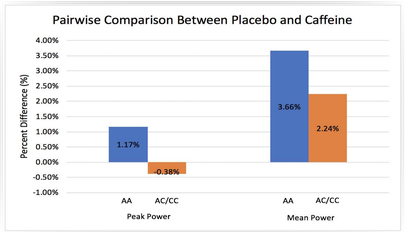

Figure 7

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