Effects of Caffeine and the -163 A>C CYP1A2, 1976 T>C ADORA2A Polymorphisms on Exhaustive Anaerobic Performance

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ABSTRACT

Individual responses to caffeine are suggested to be genetically influenced by polymorphisms of the cytochrome P450 enzymes, specifically the -163 A>C CYP1A2, for metabolism in the liver and through the adenosine receptor, 1976 T>C ADORA2A, for sensitivity of specific target cells. Individuals with the AA variant are caffeine responders, while those with the AC/CC variants are caffeine non-responders. ADORA2A TT variants demonstrate an increased sensitivity to caffeine compared to TC/CC variants. **Purpose** To examine the effect of caffeine and CYP1A2 and ADORA2A polymorphisms on anaerobic performance during exhaustive exercise. **Methods** Fifteen elite NCAA male athletes (age=20.1 ± 4.48 yrs, weight=77.4 ± 13.51 kg, height=176.7 ± 0.87 cm) participated in a double-blind study. Subjects performed two separate 90-s Wingate Tests (WAnT90) separated by two to four days on a Velotron cycle ergometer, resistance=0.05 kg•BW(kg)$^{-1}$. Subjects ingested a bolus of caffeine, 5mg•kg^{-1}BW, or a placebo (maltodextrin) one hour prior to each trial that were administered in a randomized/counterbalanced design. Anaerobic power (W•kg^{-1}), total power (W•kg^{-1}), and anaerobic capacity (W•kg^{-1}) were calculated for the 90-s and each 30-s interval. Buccal epithelial cells were collected using a mouth rinse, 0.9% NaCl, and DNA was extracted via spin columns and proteinase k. Allelic discrimination for CYP1A2 (rs762551) and ADORA2A (rs5751876) were procured via an assay and a One-Step qPCR amplification. Samples were run in duplicate, with positive and negative controls. The data was analyzed using a factorial ANOVA with repeated measures ($p > 0.05$) for each variable. **Results** Genetic analysis determined that the subject pool included (CYP1A2) 8 AA and 5 AC/CC and (ADORA2A) 1 TT and 13 TC/CC genotypes in this study. The main effect of condition, PLA versus CAF, showed significant ergogenic increase for anaerobic capacity during the first 30 seconds. The main effect of CYP1A2 genotypic variants showed significant difference for both anaerobic power and anaerobic capacity during the first 30 seconds, with AC/CC genotypes displaying an increased ergogenic effect. ADORA2A statistical analysis was not performed due to insufficient genetic variability. Anaerobic power means in watts/kg for AC/CC versus AA variants were 9.11 ± 0.49 and 8.38 ± 0.79, respectively. Anaerobic capacity means in watts/kg for CAF versus PLA were 7.59 ± 0.42 and 7.50 ± 0.34, respectively, and for AC/CC versus AA in watts/kg were 7.69 ± 0.37 and 7.52 ± 0.41, respectively. **Conclusion** Our data suggests that caffeine could increase anaerobic capacity during the first 30 seconds of exhaustive anaerobic performance, and that individuals with AC/CC variants of CYP1A2 could have increased ergogenic effects for anaerobic capacity and power for the first 30 seconds of exhaustive anaerobic performance with caffeine supplementation.

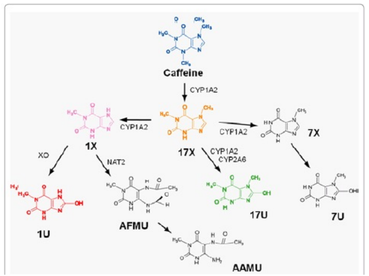

Figure 1

INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is the world's most frequently consumed and one of the most readily available psychoactive substances. Caffeine promotes wakefulness and alertness, enhances mood and cognition, and produces mild euphoria and stimulatory effects. Upon entering the body, caffeine is quickly metabolized hepatically, where the CYP1A2 enzyme from cytochrome P450 (CYP) family accounts for around 90% of caffeine's metabolism. Caffeine is rapidly broken down into three dimethylxanthine metabolites, with the main metabolite paraxanthine constituting upwards of 80% of the three dimethylxanthines (Figure 1). Paraxanthine then acts as a nonselective adenosine A_1 and A_{2A} receptor antagonist in the nervous system. Evidence suggests that caffeine offers performance improvements of about 3.2% in aerobic activity. However, anaerobic effects of caffeine has been minimally tested. Therefore, the anaerobic effects of caffeine are still largely unknown. Little testing has been conducted regarding genetic variability, specifically of CYP1A2 and ADORA2A genetic polymorphisms. Adenosine's role is to inhibit the release of brain excitatory neurotransmitters, especially dopamine, thereby resulting in fatigue. Caffeine's structure resembles adenosine and competes with it, acting as a competitive inhibitor of the A_1 and A_{2A} adenosine receptors, resulting in delayed fatigue. It is suggested that there are three gene variants of the CYP1A2 liver enzyme that control the rate at which caffeine metabolism occurs – AA, AC, and the CC polymorphisms. The AA genotypes are categorized as "fast metabolizers," which denotes that the ergogenic effects of caffeine are usually observed in the individual. The AC and CC polymorphic gene variants are considered "slow metabolizers" of caffeine, which predicts a slower and milder response to caffeine. With regards to the ADORA2A polymorphisms, individuals can be genotypically TT, TC, or CC. TT variants demonstrate an increased sensitivity to caffeine compared to TC/CC variants.

PURPOSE

To examine the effects of caffeine and specific CYP1A2 and ADORA2A genetic polymorphisms on exhaustive anaerobic performance.

METHODS

Wingate Protocol

The experimental design was done in a double-blind fashion and began with a familiarization trial. During this session, 15 participants came to the lab to collect body weight and determine cycling position. A modified 30-second WAnT of 5% bodyweight resistance was performed. Caffeine logs were kept by the participants to track caffeine intake 3 days prior to each trial. Participants completed two separate WAnT90 tests within a week of the familiarization trial, with each trial spaced out 2-4 days. Caffeine pills were created on a basis of 5 mg of caffeine per kg of bodyweight for each participant. Upon arriving at the lab, each participant ingested either their specific caffeine pill, or a maltodextrin placebo which appeared the same. The subjects waited one hour to test for caffeine to reach peak metabolism (about 1 hour). Each participant performed a 5-minute warmup to ensure blood flow throughout the body. A 2-minute rest before beginning the trial followed. Each testing trial consisted of a 90-second cycle on a Velotron bicycle with 5.0% bodyweight resistance. Power data was collected by the WAnT90 computer program during the trial. Once the test was complete, there was a 10-minute cooldown for recovery.

CYP1A2 and ADORA2A Protocol

Buccal epithelial cell samples were collected from each subject at the familiarization trial. A 0.9% NaCl saline mouth rinse was used, and each participant swished it in his mouth for one minute. To isolate DNA, the the Qiagen DNA isolation kit was used. Protein kinase K was used to lyse and break down cellular structures so that the DNA would become accessible for further examination. To analyze individuals' CYP1A2 and ADORA2A genotypes, a TaqMan® Genotyping Master Mix was used. The samples plated either contained the TaqMan® SNP Assay for the CYP1A2 gene or the ADORA2A gene, as well as the participants' DNA, which was normalized to 5 ng/µL. Then, a quantitative polymerase chain reaction (qPCR) protocol followed and consisted of 40 thermocycles for amplification with a One-Step qPCR. This reaction allowed for an allelic discrimination plot to be generated. From this plot, the genotype of the individuals' CYP genes were determined.

RESULTS

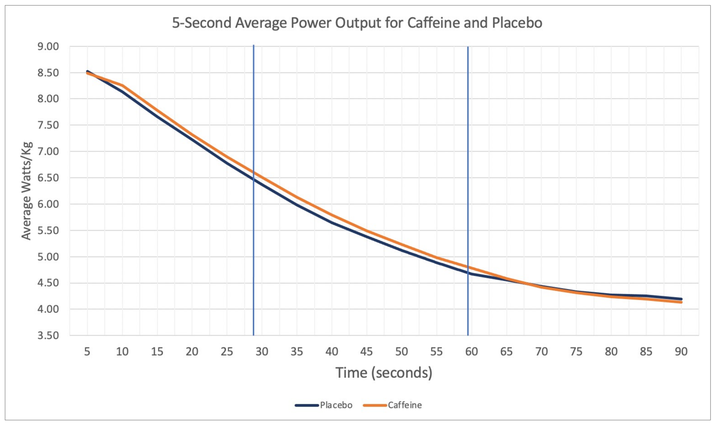

Figure 1

Genotyping:
CYP1A2: 8 AA and 5 AC/CC individuals
ADORA2A: 1 TT and 13 TC/CC individuals*

The data was analyzed using a 2 (condition) x 2 (CYP) ANOVA with repeated measures ($p>0.05$):

- The main effect of condition, PLA versus CAF, showed significant difference for anaerobic capacity during the first 30 seconds ($p < 0.05$). (Figure 4)
- The main effect of CYP1A2, AA or AC/CC, showed significant difference for both anaerobic power and anaerobic capacity during the first 30 seconds ($p < 0.01$).(Figure 2) (Figure 3)
- There was no significant difference for the condition or the CYP for either the middle 30 seconds or the final 30-seconds.

*ADORA2A was not run for statistical analysis at this time due to insufficient numbers in allelic distribution.

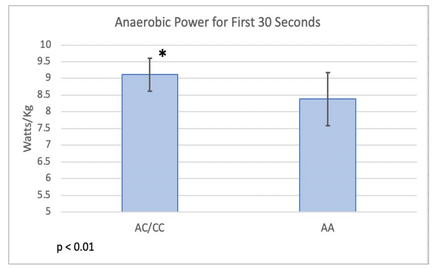

Figure 2

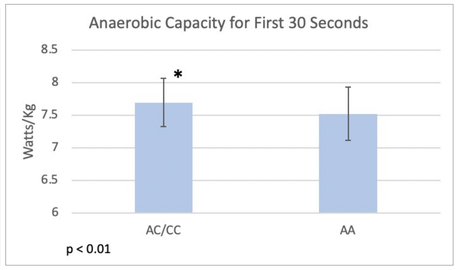

Figure 3

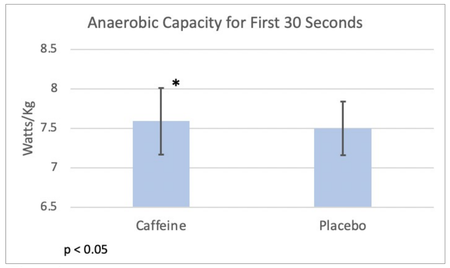

Figure 4

CONCLUSION

Data suggests that there is a positive ergogenic effect on anaerobic capacity from caffeine during the first 30 seconds of exhaustive anaerobic performance. Data also suggests that AC/CC genotypes increase anaerobic power and anaerobic capacity compared to AA genotypes during the first 30 seconds.

Figure 1 displays the 5-second averages for both the caffeine and placebo trials, with blue lines indicating each 30-second interval. Only during the first 30-second interval was any significance determined. Total anaerobic power for the full 90 seconds after caffeine ingestion was close to significantly increasing performance ($p = 0.116$). Our results indicate that there is ergogenic benefits for consuming caffeine when performing exhaustive exercise for up to 30 seconds. Anaerobic capacity is equivalent to the mean power output over a set time, so caffeine supplementation significantly increases individuals' average power output for up to 30 seconds. Anaerobic power is the peak power output for a set time. Therefore, our data suggests genotypic AC/CC individuals can output a greater peak wattage/kg compared to AA individuals during the first 30 seconds of performance, as well as have greater mean power output (anaerobic capacity) compared to AA individuals for the first 30 seconds of performance. Our genetically related results were unexpected, as AA individuals are fast metabolizers of caffeine, which should predict an increased effect with caffeine. This could be attributed to the influence of ADORA2A polymorphisms, which we were unable to analyze due to a lack of genetic variability among participants. A colleague is investigating the pharmacodynamics of caffeine, which could help determine peak metabolism of caffeine. Our one hour wait period could have caused AA individuals to pass the point of peak metabolism. Further experiments will help to determine optimal timing of caffeine ingestion to time of performance.