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Abstract

Purpose: Many studies have examined the effect of caffeine on exercise performance, but findings have not always been consistent. The objective of this study was to determine whether variation in the *CYP1A2* gene, which affects caffeine metabolism, modifies the ergogenic effects of caffeine in a 10-km cycling time trial. **Methods:** Competitive male athletes (n=101; age: 25 ± 4 years) completed the time trial under three conditions: 0, 2 or 4 mg of caffeine per kg body mass, using a split-plot randomized, double-blinded, placebo-controlled design. DNA was isolated from saliva and genotyped for the -163A>C polymorphism in the *CYP1A2* gene (rs762551). **Results:** Overall, 4 mg/kg caffeine decreased cycling time by 3% (mean ± SEM) versus placebo (17.6 ± 0.1 vs. 18.1 ± 0.1 min, p = 0.01). However, a significant (p <0.0001) caffeine-gene interaction was observed. Among those with the AA genotype, cycling time decreased by 4.8% at 2 mg/kg (17.0 ± 0.3 vs. 17.8 ± 0.4 min, p = 0.0005) and by 6.8% at 4 mg/kg (16.6 ± 0.3 vs. 17.8 ± 0.4 min, p < .0001). In those with the CC genotype, 4 mg/kg increased cycling time by 13.7% versus placebo (20.8 ± 0.8 vs. 18.3 ± 0.5 min, p = 0.04). No effects were observed among those with the AC genotype. **Conclusion:** Our findings show that both 2 and 4 mg/kg caffeine improve 10-km cycling time, but only in those with the AA genotype. Caffeine had no effect in those with the AC genotype and diminished performance at 4 mg/kg in those with the CC genotype. *CYP1A2* genotype should be considered when deciding whether an athlete should use caffeine for enhancing endurance performance.

Key Words: EXERCISE, GENES, CYCLING, TIME TRIAL, ERGOGENIC, NUTRIGENOMICS

INTRODUCTION

Caffeine is frequently used by athletes because of its reported performance-enhancing or ergogenic effects [1-15]. Numerous studies have investigated the effect of supplemental caffeine on aerobic endurance performance, and while most reliably show performance enhancement with caffeine use [1-15], there is considerable inter-individual variability as to the magnitude of these effects [16-20]. For example, in a study of caffeine effects in runners, Graham *et al*. [5] reported that endurance benefits were associated with caffeine supplementation overall, but the magnitude of the improvements ranged from 5% to 87% and 10% to 156% in running and cycling time-to-exhaustion trials, respectively. Similarly, Doherty *et al*. [6] found that during treadmill running time-to-exhaustion, 9 out of 14 subjects improved, while 5 subjects did not, during the caffeine versus placebo trials. Wiles *et al*. [7] also found that the mean improvement during a 1-kilometer (km) cycling time trial was 3% overall under caffeine conditions, but individual results ranged from one subject performing worse to another improving their performance by 6%.

In contrast to most caffeine-performance studies, no ergogenic effect of caffeine was reported by Roelands *et al*. [19] in a study involving trained male cyclists. The authors concluded that inter-individual differences in response to caffeine might be responsible for the lack of overall performance improvement, as 50% of subjects improved while 50% worsened, in the caffeine compared to the placebo trial. Similarly, Skinner *et al*. [16] found no effect of caffeine at 2, 4, or 6 mg/kg versus placebo in a rowing time trial, which may have been due, in part, to the large variation in individual response to caffeine. The authors noted that this consideration is often overlooked in caffeine performance studies, and due to infrequent reporting of individual data it is difficult to determine the extent to which variation in responses may be occurring. The performance of some individuals is often in stark contrast to the average findings reported, which may conclude beneficial, detrimental or no effect of caffeine on performance.

Studies that report on the effects of caffeine on performance have been inconsistent despite having similar study designs, subjects and dose of caffeine. These inconsistencies might be due, in part, to inter-individual differences in caffeine metabolism or caffeine response. Over 95% of caffeine is metabolized by the CYP1A2 enzyme, which is encoded by the *CYP1A2* gene, and is involved in the demethylation of caffeine into the primary metabolites paraxanthine, theophylline and theobromine [21]. The -163A>C (rs762551) single nucleotide polymorphism (SNP) has been shown to alter CYP1A2 enzyme inducibility and activity [22, 23], and has been used to categorize individuals as 'fast' or 'slow' metabolizers of caffeine. Individuals with the AC or CC genotype (slow metabolizers) have an elevated risk of myocardial infarction [24], hypertension [25], and pre-diabetes [26] with increasing caffeinated coffee consumption, whereas those with the AA genotype show no such risk. In addition, a few studies have shown that the rate of caffeine metabolism could also have implications for sports performance, but the findings remain equivocal [12, 27-29].

The objective of this study was to determine the effects of low (2 mg/kg) or moderate (4 mg/kg) doses of caffeine supplementation on endurance performance, and whether variation in the *CYP1A2* gene modifies these effects among competitive male athletes recruited from a variety of sports.

METHODS

Subjects and Recruitment. Recruitment was carried out at the University of Toronto, Ryerson and York University campuses, the Canadian Sport Institute of Ontario, local running/triathlon clubs and training gyms using posted flyers. A standardized email with study details and contact information was also sent to head coaches, program directors of sports teams or clubs, and some professional sport organizations with eligible athletes. Ethics approval was obtained from the University of Toronto Institutional Review Board, and the study was registered with clinicaltrials.gov (NCT 02109783). All subjects provided written informed consent, and were informed that they could terminate their participation in the study at any time.

A total of 113 competitive male athletes from a variety of sports participated in the present study. Subjects were recruited from a wide range of sports that could be classified into three categories: endurance (e.g. marathon, triathlon, cycling, cross-country skiing), power (e.g. boxing, volleyball, dragon-boat, powerlifting) or mixed (e.g. soccer, rugby, basketball, swimming). All participants were training and/or competing for ≥ 8 hr per week, 9 out of 12 months per year, and for at least 3 years in their given sport. Eight athletes dropped out of the study due to a sport-related injury (n=3), school or work demands (n=2), unwillingness to abstain from caffeine (n=2), or relocation (n=1). Four subjects were excluded because of incomplete data. The remaining 101 athletes had a mean ± SD age of 25 ± 4 years and body mass of 81.3 ± 12.4 kg.

Experimental Design. A split-plot randomized, double-blinded, placebo-controlled study design was used. Subjects completed 4 visits (~90-120 min each) that were approximately 1 week apart, in the exercise laboratory at the Goldring Centre for High Performance Sport at the University of Toronto. During the first laboratory visit, each subject had descriptive and anthropometric data collected, completed a maximal aerobic capacity test (VO_2peak) and completed a questionnaire on general health, caffeine intake habits, and sport history. Subjects also provided a saliva

sample for DNA analysis. Testing took place on weekdays and weekends, and the treatment visits were scheduled at the same time of day, every 7 days, for each athlete. Participants were instructed to maintain their regular diet and sleeping habits, avoid strenuous activity 48 hours before each visit, and abstain from caffeine one week prior to the first visit and for the duration of the data collection (4 weeks total). To ensure dietary consistency prior to testing across all visits, participants were advised on their first visit to consume meal(s) that could be easily replicated for all subsequent treatment visits. Participants were also reminded of their required meal composition by email or text message one day prior to each visit. On treatment visits 2-4, subjects were randomly assigned to ingest capsules containing either anhydrous caffeine (American Chemicals Ltd, Montreal, Quebec) at 2 or 4 mg/kg body mass or placebo (PLAC). The PLAC (dextrose) capsule was tasteless, and had the same volume and color as the caffeine. After ingestion, the subjects sat quietly (completing questionnaires or using e-devices) in the laboratory for 25 minutes before commencing their warm-up and four exercise tests. Blood pressure and heart rate were measured after capsule ingestion and 3 minutes of sitting quietly, and again 20 min later, just prior to warm up. This protocol was repeated three times; one for each treatment (0, 2 or 4 mg/kg caffeine).

Parameters of Assessment.

Before testing, athletes were led through a brief standardized warm-up that consisted of light cycling and stretching for approximately 7 minutes. Physical tests were conducted in a standard order to minimize fatigue: 1) Vertical Jump 2) Handgrip 3) Wingate 4) 10-km Cycling Time Trial (TT). Only the results of the 10-km cycling TT are reported here.

Anthropometry. Height was measured with a Harpenden stadiometer (Holtain, Crymych, UK) and body mass was measured by an electronic floor scale (AND FW-150K; Tokyo, Japan). Total body fat % was measured by BC-558 IronMan Segmental Body Composition Monitor (Tanita, Arlington Heights, IL, USA).

Maximal Exercise Test (VO_2peak). Subjects began the test at a work rate of 50 Watts (W) on a mechanically weighted and braked ergometer (Monark Ergomedic 839E), with load increases of 50 W each minute for the first two minutes, then 25 W each minute thereafter until volitional exhaustion. Gas exchange was measured by a portable metabolic system (Cortex Metamax 3B®), and maximal oxygen uptake (VO_2peak) was defined as the highest 1-minute oxygen value obtained during the test. VO_2peak power (W) was calculated by measuring the power output (W) at VO_2peak, and end power W_{power} was calculated as the power output (W) at volitional fatigue. Heart rate was monitored using a Polar Heart Rate Monitor (Lake Success, NY).

Time Trial. Subjects commenced the 10-km cycling TT (last exercise) when blood lactate levels reached <2.5 mmol/L from the prior Wingate test. The TT was conducted by setting the Ergomedic 839 E stationary bike to a constant resistance or power output, and each subject cycled 10-km at the specified resistance (Watts). Resistance was set at 65% W_{power} for all subjects as calculated from the VO_2peak test, which was the equivalent of 65-69% VO_{2peak} (varying between subjects but identical % used within each subject for all three treatment visits). The on-board computer automatically controls the degree of resistance by applying varying amounts of braking force on the belt. The computer of the stationary bike calculates the speed of travel based on the cadence of pedalling (RPM), where a faster cadence would result in a faster speed. The 10-km TT requires 1,667 rotations (6 m per rotation) to be completed; therefore, the power output did not affect the speed of the bike. Speed was altered only by how fast the subject pedalled (cadence). Therefore, different cadences (RPM) would result in different completion

times for the 10-km TT. Subjects were blinded to time, speed and heart rate, but were able to see distance traveled. Water was available ad libitum throughout the TT. Heart rate was monitored throughout the test using a Polar Heart Rate Monitor (Lake Success, NY). Subjects estimated their Ratings of Perceived Exertion (RPE) on the basis of Borg's rating scale (score ratings from 6-20, where 6 is no exertion, and 20 is extremely difficult) at 5-km and 9-km.

Genotyping. Saliva samples were collected on visit 1 using the Oragene ON-500 kit for DNA isolation using standard procedures, as previously described [30]. Genotyping of the rs762551 SNP in the *CYP1A2* gene was conducted using the Sequenom MassArray platform, as we have described previously [30]. Since there is evidence of a difference in enzyme activity between the three *CYP1A2* genotypes [22, 23], we grouped individuals into AA (fast), AC (heterozygous slow) and CC (homozygous slow) for all analyses.

Statistical Analyses. Data were analyzed using the SAS statistical package (SAS 9.4, SAS Institute Inc., USA), and are presented as mean ± SEM unless stated otherwise. Descriptive data (height, body mass, age, body fat, VO_2peak [$L \cdot min^{-1}$], VO_2peak [$ml \cdot kg^{-1} \cdot min^{-1}$] dietary caffeine or caffeine used for sport, sport type distribution) were compared between genotypes using analysis of variance (ANOVA) or for sport type, using Chi-Square. Body mass was log-transformed before analysis, as it was not normally distributed. Using a classical split-plot design, the between subject variance was used to compare mean cycling times across the three genotypes while the within subject variance was used to compare placebo and the two caffeine doses. The order of the three visits was randomized across the subjects and visit was included as a co-variate in all analyses. Randomization was done using balanced permutations blocked by time of entry (randomization.com). The outcome variable was 10-km TT time, and the initial analysis included the three predictor variables caffeine, gene, visit, along with the three 2-factor interactions and the one 3-factor interaction. After identifying a significant caffeine-gene

($p<0.0001$) interaction, each genotype was analyzed separately. This model was also used to assess RPE and HR between genotypes and within subjects between visits and caffeine treatments. The main effect of caffeine was assessed across all genotypes combined, which left two predictor variables: caffeine and visit, and the caffeine-visit interaction, with TT time to completion as the outcome variable. Post-hoc Tukey adjustments for multiple comparisons were performed for all analyses. All p-values are two-tailed and $p < 0.05$ was used as the threshold for significance. Effect Sizes (ES) are presented as standardized differences between caffeine treatments (all subjects combined or for individual genotypes) using Cohen's $d = (M_2 - M_1) / SD_{pooled}$, where $SD_{pooled} = \sqrt{((SD_1^2 + SD_2^2) / 2)}$ [31]. Cohen [31] suggested that 0.2 be considered a 'small', 0.5 represents a 'medium' and 0.8 a 'large' effect size. For significant genotype and treatment p-values, the analysis of the effect of caffeine dose on the mean 10-km TT time was completed with and without an adjustment for the visit variable, to establish whether visit was a confounder. This was completed for the main effect of caffeine for all subjects, as well as the effect of caffeine within each of the three genotypes. Sample size was determined by power analysis calculations using a power of 0.8, and a medium effect size of 0.5. A power calculation based on two caffeine doses and three genotypes revealed that a sample size of 110 athletes will provide sufficient power for our analysis, based on a potential subject drop out rate of 10% [31].

RESULTS

Subject Characteristics. Of the 101 participants, 49% (n = 49) were homozygous for the A allele (AA), 43% (n = 44) were heterozygous (AC), and 8% (n = 8) were homozygous for the C allele (CC). These distributions are in Hardy-Weinberg equilibrium, and similar to frequencies reported previously in some other populations [22, 24]. The rs762551 polymorphism in the *CYP1A2* gene was initially used to identify fast and slow metabolizers of caffeine. We

discovered that another SNP in *CYP1A2,* rs2472300, is in 100% linkage disequilibrium with rs762551. As such, either polymorphism can be used to identify fast or slow metabolizers of caffeine. For rs2472300, GG corresponds to fast metabolizers whereas GA and AA are considered slow metabolizers. For rs762551, AA corresponds to fast metabolizers whereas AC and CC are considered slow metabolizers. In the present study, we genotyped subjects for both the rs2472300 and rs762551 SNPs and found 100% concordance, but we report the results for rs762551 because it is the one more commonly reported [12, 22-26, 29].

Descriptive characteristics of the three genotypes are shown in Table 1. There were no significant differences between the three genotypes for age, height, body fat, VO_2peak ($L \cdot min^{-1}$), VO_2peak ($ml \cdot kg^{-1} \cdot min^{-1}$), dietary caffeine, caffeine used for sport or percent distribution of sport type (endurance, power or mixed; X^2 [4, N = 101] = 3.31). The breakdown for sport type for all participants was as follows: endurance 42% (e.g. running, cycling, rowing); power 42% (e.g. baseball, powerlifting, boxing); and mixed 16% (e.g. basketball, rugby, hockey).

Time Trial Performance.

All Subjects. The average 10-km TT times (n = 101) under the three treatments (0, 2 or 4 mg/kg caffeine) are shown in Figure 1. There was a significant (p = 0.04) main effect for treatment (2 or 4 mg/kg caffeine vs placebo) for all subjects, where 4 mg/kg caffeine decreased 10-km TT time by 3% (0.5 min) compared to placebo (17.6 ± 0.3 vs. 18.1 ± 0.1 min, p = 0.01). There was no significant difference between 2 mg/kg and either 4 mg/kg caffeine or placebo.

By Genotype.

When subjects were stratified by caffeine dose (0, 2, 4 mg/kg) and genotype (Figure 2) there was a significant overall difference between genotypes (p = 0.002), as well as a caffeine-gene

($p<0.0001$) interaction. Thus, the three genotypes were analyzed individually to determine the effects of caffeine within each genotype.

AA genotype (fast metabolizers). Among those with the AA genotype, the caffeine effect remained significant, where 2 mg/kg caffeine decreased TT time by 4.8% (0.8 min) compared to placebo (17.0 ± 0.3 vs. 17.8 ± 0.4 min, $p = 0.0005$), and by 6.8 (1.2 min) in 4 mg/kg compared to placebo (16.6 ± 0.3 vs. 17.8 ± 0.4 min, $p <0.0001$), but no difference was observed between 2 and 4 mg/kg caffeine.

AC genotype (slow metabolizers). In those with the AC genotype, there was no caffeine effect on TT performance for any of the treatments (18.6 ± 0.4, 18.4 ± 0.5, 18.0 ± 0.5, for 0, 2 and 4 mg/kg, respectively; $p = 0.43$).

CC genotype (slow metabolizers). Among those with the CC genotype, 4 mg/kg caffeine increased cycling time by 13.7% (2.5 min) compared to placebo (20.8 ± 0.8 vs. 18.3 ± 0.5 min, $p = 0.04$), but no difference was observed between 2 mg/kg and either 4 mg/kg caffeine or placebo.

Change in TT time: placebo vs 2 mg or 4mg/kg caffeine. Figure 3 shows the average change in TT time (mean ± SEM) to completion between the (A) 2 mg/kg and (B) 4 mg/kg caffeine dose, compared to placebo. In Figure 3A (2 mg/kg vs placebo), there were no differences between any of the genotypes. Figure 3B shows a significant ($p = 0.001$) overall difference between genotypes, such that those with the CC genotype had the greatest change in 10-km time (although a worsening of performance with caffeine) compared to changes in time in the opposite direction in those with the AA (-2.5 ± 1.0 min vs 1.2 ± 0.3 $p<0.0001$) and AC (-2.5 ± 1.0 min vs 0.6 ± 0.4 min, $p = 0.0015$) genotypes, respectively.

TT Performance Scatterplot by Genotype. Figure 4 shows individual data points representing 10-km time to completion for placebo (x-axis) and either (A) 2 mg/kg or (B) 4 mg/kg, (y-axis), for all subjects by genotype (AA, AC, CC). Data points below the line indicate faster times with

caffeine. For those with the AA genotype, 35 (71%) and 40 (82%) out of 49 subjects performed better during 2 or 4 mg/kg, respectively, compared to placebo. In those with the AC genotype, 26 (59%) and 28 (64%) out of 44 subjects performed better during 2 or 4 mg/kg caffeine, respectively, compared to placebo. In those with the CC genotype, 2 (25%) and 1 (12%) out of 8 subjects performed better during 2 or 4 mg/kg respectively, compared to placebo.

RPE and HR. At 5-km, those with the AA genotype reported a 3% lower RPE in the 4 mg/kg TT compared to placebo (14.3 ± 0.3 vs 14.8 ± 0.2, p = 0.03), but there was no difference between 2 mg/kg (14.5 ± 0.3) and either 4 mg/kg caffeine or placebo. There were no differences in those with the AC genotype (15.1 ± 0.3, 15.5 ± 0.3, 15.0 ± 0.3) or CC genotype (14.1 ± 0.6, 14.3 ± 0.6, 15.5 ± 0.3) between any of the TTs at 0, 2 or 4 mg/kg caffeine, respectively. At 9-km there were no differences in RPE between any of the treatments within any of the genotypes. Heart Rate (HR) analysis was determined in those with the AA (n=46), AC (n=42) and CC (n=6) genotypes. In those with the AA genotype, there were no significant differences in HR (mean ± SEM) between any of the doses (167 ± 1, 169 ± 1, 168 ± 1 bpm for 0, 2 or 4 mg/kg caffeine, respectively). In those with the AC genotype, there was a 2.5% (4 bpm) increase in HR in 4 mg/kg compared to 2 mg/kg caffeine and placebo, respectively (171 ± 2 vs 167 ± 2 bpm, p = 0.007 and 167 ± 2 bpm, p = 0.005). In those with the CC genotype, there was a 2% (3 bpm) decrease in HR in those taking 4 mg/kg caffeine compared to both placebo and 2 mg/kg (160 ± 5 vs 157 ± 5 bpm, p = 0.03; 160 ± 5 vs 157 ± 5 bpm, p= 0.05), respectively.

Effect Size (ES). The main effect for caffeine (n = 101) in the 10-km TT at the 4 mg/kg dose, resulted in a 3% (0.5 min) improvement and small ES, d =0.27, compared to placebo. However, in those with the AA genotype (n = 49) the 4.8% (0.8 min) improvement with 2 mg/kg and the 6.8% (1.2 min) improvement with 4 mg/kg, both correspond to a medium ES: d = 0.4 and d =

0.63, respectively. In those with the CC genotype, the 13.7% impairment in performance in 4 mg/kg vs placebo resulted in a very-large ES, d =1.3.

Treatment Blinding. We collected responses from 86 subjects post-TT, who were asked whether or not they thought they had consumed caffeine. Out of 172 caffeine trials, 31% (54) were correctly identified as caffeine-containing. Among the other 118 caffeine trials, 81% (96) reported 'no caffeine' and 19% (22) reported 'maybe caffeine'. Only 3% (3) of subjects correctly identified all three trials (i.e. 2 caffeine, 1 placebo).

Familiarization. A learning or visit effect due to familiarization with cycling on the Monark bike for the three treatment visits (plus cycling VO_2peak test) was expected in this group of athletes, where less than 6% were experienced cyclists. Although we observed well-balanced allocation of the three doses of caffeine across all three visits where χ^2 (4, N = 101) = 2.01, p = 0.73, we assessed the effect of visit within each genotype. In those with the AA genotype, TT time decreased across visits, likely as a learning or familiarization effect. However, TT cycling time also decreased within each visit as caffeine dose increased from 0 mg to 2 mg/kg to 4 mg/kg, where ~33% of subjects would have ingested one of the three caffeine doses at each particular visit. Therefore, at each visit, each group consisting of one third of the 101 total subjects improved their performance in a dose dependent manner after caffeine ingestion from 0 mg to 2 mg/kg to 4 mg/kg. Table 2 shows the TT time and caffeine dose by genotype with and without adjusting for visit. When analyzing the effect of caffeine and visit in each genotype individually, the caffeine effect remained significant in both the AA ($p < 0.0001$) and CC ($p = 0.04$) genotypes. The predictive power (R^2) dropped from 0.85 in the model with all subjects (not shown) to 0.78 in the model with AA genotypes and 0.80 in the model with CC genotypes. When visit was not included in the model (Table 2, model 1), the caffeine effect remained significant in the AA genotype ($p < 0.0001$), but decreased the predictive power of the model ($R^2 = 0.70$).

However, in those with the CC genotype, the caffeine effect was no longer significant, and R^2 decreased to 0.56.

DISCUSSION

The current study examined the effects of caffeine and a genetic modifier of caffeine metabolism, *CYP1A2* genotype, on 10-km cycling TT performance in competitive male athletes after ingestion of caffeine at 0, 2 and 4 mg/kg body mass. Our results indicate that in the total population caffeine is ergogenic to endurance cycling performance, with a 3% improvement in TT time at 4 mg/kg, but not at 2 mg/kg, which is consistent with previous studies using similar doses [2, 9]. However, we observed a significant caffeine-gene interaction where the improvements in performance were seen with both 2 and 4 mg/kg caffeine, but only in those with the AA genotype who are 'fast' metabolizers of caffeine. In that group, the 6.8% improvement in cycling time at 4 mg/kg, is greater than the 2-4% mean improvement seen in approximately 30 cycling TTs studies using similar doses [2, 8, 9, 14, 15, 18, 32]. The improvement in performance that we observed in the entire population at 4 mg/kg corresponds to a small effect size, d =0.27, but in those with the AA genotype the effect corresponds to a medium effect size d = 0.63. Contrary to the beneficial effects we observed among those with the AA genotype, we found that 4 mg/kg caffeine impaired performance by 13.7% in those with the CC genotype who are 'slow' metabolizers of caffeine, and this corresponds to a very large effect size, d =1.3. We found no effect of either dose in those who have the AC genotype.

Most studies on caffeine and performance do not explore the basis for the inter-individual variation in response, which has been well-documented in several studies [5-7, 14, 19]. For example, Jenkins *et al*. [14] examined the effects of caffeine on exercise performance in thirteen cyclists, and the inter-individual range for performance change with caffeine at 1, 2 or 3 mg/kg

compared with placebo was –7.9% to 17.8%. Although 11 of 13 cyclists benefited from the 3 mg/kg dose, the authors noted that "the mean performance outcome did not reach statistical significance due to two "non-responders" strongly influencing the mean and SEM, in addition to 8 of the 13 subjects performing worse on at least one caffeine condition versus placebo. Similarly, Paton *et al*. [11] found that caffeinated (~3-4 mg/kg) chewing gum improved overall performance in 20 male and female cyclists, but only 13 (65%) of the cyclists were considered 'positive responders' while 5 (20%) experienced 'negative' responses and the remaining 2 (15%) experienced no observable effect on cycling performance. The authors speculated that this variation in response may be related to differences in the rate of caffeine metabolism or absorption between individuals [11].

Acute caffeine ingestion has been shown to alter RPE, where effort may be greater under caffeine conditions, yet it is not perceived as such [1, 33]. Consistent with other studies [1, 33], our results showed a 3% decrease in RPE for the AA genotype at 5km after taking 4 mg/kg caffeine, which coincides with the group that had the fastest 10-km TT time. Those with the CC genotype taking 4 mg/kg had a non-significant increase in RPE, which is consistent with the impaired performance in that group. Our findings suggest that caffeine does not lower RPE in all individuals. Similarly, a recent study by Green *et al*. [34] showed that when subjects were instructed to cycle at specific RPE (effort) levels under caffeine conditions, the higher perceived intensity did not necessarily result in greater work and improved performance in all subjects equally. The authors noted that individual responses to the caffeine may explain their unexpected findings.

In the present study, only those with the AA genotype who are fast metabolizers of caffeine benefited from caffeine during the 10-km TT. There is some evidence that extended periods of blocked adenosine receptors may be detrimental to performance [36], and this may explain the

lack of benefit or diminished performance in slow metabolizers. Slower clearance of caffeine, and longer caffeine build-up in slow metabolizers has been associated with increased blood pressure [25], and this vasoconstriction may also have effects on both blood flow to the heart and muscles [35]. Resting myocardial blood flow does not appear to be affected by caffeine ingestion, but exercise-induced myocardial blood flow has been shown to decrease after caffeine ingestion [35]. Under exercise conditions the expected adenosine-mediated coronary vasodilation and subsequent increase in myocardial blood flow to match augmentation in cardiac work, is likely impaired by caffeine and could explain the impaired performance among slow metabolizers [35, 36]. It has also been postulated that caffeine metabolites, such as paraxanthine, may have ergogenic properties and would be generated more quickly in fast metabolizers, thereby providing benefits sooner than in slow metabolizers [12]. The initial ergolytic effects of impaired adenosine-mediated vasodilation experienced by fast metabolizers may be outweighed by their ability to expedite the production of these metabolites, which may be the source of ergogenicity.

Our findings are consistent with a previous study by Womack *et al*. [12] who observed a caffeine-gene interaction and improved TT cycling performance with caffeine only in those with the AA genotype. In contrast, previous studies either did not observe any impact of the *CYP1A2* gene on caffeine ergogenicity [27, 28], or reported benefits only in slow metabolizers [29]. Pataky *et al*. [29] reported an improvement in those with the AC genotype compared to the AA genotype after caffeine ingestion, however, the 3-km TT performed in that study was a much shorter duration than in the present study or in the previous study [12]. Furthermore, that study [29] did not include any subjects with the CC genotype, which is the group that we found to have impaired performance after 4 mg/kg caffeine. Algrain *et al*. [28] found no effect of 255 mg

caffeine on a 15-minute cycling performance trial, and no modifying effect of *CYP1A2* genotype. However, this dose of caffeine was likely too low to observe any effect, since most previous studies report an effect only at higher doses. That study also had a small sample size of 20 subjects (AA genotype, n=10; C-allele carriers, n = 9), did not differentiate between AC and CC genotypes among C-allele carriers, and included both males and females, which may have been a confounder due to potential gender differences in caffeine response by genotype [37]. Similarly, no effects of *CYP1A2* on caffeine ergogenicity were observed in the study by Salinero *et al*. [27], but the very small sample size of only 10 subjects with the AA genotype, compared to 49 subjects with this genotype in the present study, make it unlikely that any significant findings would be detected. Importantly, that study used a 30-sec Wingate test, which is a measure of power or anaerobic capacity, and is not a valid measure of endurance [27]. Consistent with this notion, a meta-analysis of caffeine and exercise performance [4] showed that larger effect sizes with caffeine supplementation were more often reported in trials of longer duration. Therefore, the unmasking of the effects of genotype on performance may occur during exercise of longer duration and during an accumulation of fatigue, where caffeine often provides its greatest benefits, and where the adverse effects to slow metabolizers are more likely to manifest. And as previously mentioned, this may improve performance by allowing for a greater accumulation of the potentially ergogenic caffeine metabolites, in fast metabolizers.

Two concerns often raised in caffeine-performance studies using cycling time trial protocols are 1) a learning effect and 2) the caffeine-placebo effect. To address the issue of familiarization, we included a visit variable in our statistical model, to control for potential confounding due to a learning effect. Although performance improved with each successive visit, the improved performance with caffeine in those with the AA genotype occurred regardless of the order of treatment and the findings were the same with and without adjusting for visit. The caffeine-

placebo effect can introduce a psychological factor [32] outside of the expected physiological effect, when a subject is not blinded to the treatment. Treatment blinding in the present study was successful with less than one third of caffeine trials identified correctly. Although a placebo benefit has been reported to occur in subjects who believe they have ingested caffeine [32, 38], this would not explain the benefits seen only in those with the AA genotype.

Our results also confirm the ergogenicity of lower caffeine doses (2-4 mg/kg) as previously reported [9, 13], but only within a specific genetic subset of individuals. Lower caffeine doses are more desirable in order to avoid the potential adverse side effects of higher doses (6-9 mg/kg), such as sleep disturbances [39], especially for athletes training or competing at night, as well as other adverse effects such as anxiety and agitation [40].

Although the results from the present study suggest a potential role of *CYP1A2* genotype in influencing the ergogenic response of caffeine in competitive athletes from a variety of sports, care should be taken in extrapolating these findings to female, non-athletic or older populations. It is unknown if there is a similar genetic influence for other modes of exercise of high-intensity or short-duration, or whether other polymorphisms in *CYP1A2* or other genes involved in the response to or metabolism of caffeine may modify the effects of caffeine during exercise.

In summary, we found that caffeine improves endurance performance at a dose of 2 and 4 mg/kg for fast metabolizers of caffeine who have the *CYP1A2* AA genotype (rs762551). Among the slow metabolizers, there is either no effect (AC genotype) or impaired performance (CC genotype) under the caffeine conditions in this study. These results highlight the importance of considering *CYP1A2* genotype when deciding whether athletes should use caffeine as an ergogenic aid to improve endurance performance.

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REFERENCES

1. Doherty, M. and P.M. Smith, Effects of caffeine ingestion on rating of perceived exertion during and after exercise: a meta-analysis. Scand J Med Sci Sports, 2005. **15**(2): p. 69-78.
2. Ganio, M.S., et al., Effect of caffeine on sport-specific endurance performance: a systematic review. J Strength Cond Res, 2009. **23**(1): p. 315-24.
3. Hodgson, A.B., R.K. Randell, and A.E. Jeukendrup, The metabolic and performance effects of caffeine compared to coffee during endurance exercise. PLoS One, 2013. **8**(4): p. e59561.
4. Doherty, M. and P.M. Smith, Effects of caffeine ingestion on exercise testing: a meta-analysis. Int J Sport Nutr Exerc Metab, 2004. **14**(6): p. 626-46.
5. Graham, T.E. and L.L. Spriet, Performance and metabolic responses to a high caffeine dose during prolonged exercise. J Appl Physiol (1985), 1991. **71**(6): p. 2292-8.
6. Doherty, M., et al., Caffeine is ergogenic after supplementation of oral creatine monohydrate. Med Sci Sports Exerc, 2002. **34**(11): p. 1785-92.
7. Wiles, J.D., et al., The effects of caffeine ingestion on performance time, speed and power during a laboratory-based 1 km cycling time-trial. J Sports Sci, 2006. **24**(11): p. 1165-71.
8. Skinner, T.L., et al., Coinciding exercise with peak serum caffeine does not improve cycling performance. J Sci Med Sport, 2013. **16**(1): p. 54-9.
9. Desbrow, B., et al., The effects of different doses of caffeine on endurance cycling time trial performance. J Sports Sci, 2012. **30**(2): p. 115-20.
10. Ganio, M.S., et al., Effect of ambient temperature on caffeine ergogenicity during endurance exercise. Eur J Appl Physiol, 2011. **111**(6): p. 1135-46.

11. Paton, C., V. Costa, and L. Guglielmo, Effects of caffeine chewing gum on race performance and physiology in male and female cyclists. J Sports Sci, 2015. **33**(10): p. 1076-83.

12. Womack, C.J., et al., The influence of a CYP1A2 polymorphism on the ergogenic effects of caffeine. J Int Soc Sports Nutr, 2012. **9**(1): p. 7.

13. Talanian, J.L. and L.L. Spriet, Low and moderate doses of caffeine late in exercise improve performance in trained cyclists. Appl Physiol Nutr Metab, 2016. **41**(8): p. 850-5.

14. Jenkins, N.T., et al., Ergogenic effects of low doses of caffeine on cycling performance. Int J Sport Nutr Exerc Metab, 2008. **18**(3): p. 328-42.

15. Graham-Paulson, T., C. Perret, and V. Goosey-Tolfrey, Improvements in Cycling but Not Handcycling 10 km Time Trial Performance in Habitual Caffeine Users. Nutrients, 2016. **8**(7).

16. Skinner, T.L., et al., Dose response of caffeine on 2000-m rowing performance. Med Sci Sports Exerc, 2010. **42**(3): p. 571-6.

17. Hunter, A.M., et al., Caffeine ingestion does not alter performance during a 100-km cycling time-trial performance. Int J Sport Nutr Exerc Metab, 2002. **12**(4): p. 438-52.

18. Bortolotti, H., et al., Performance during a 20-km cycling time-trial after caffeine ingestion. J Int Soc Sports Nutr, 2014. **11**: p. 45.

19. Roelands, B., et al., No effect of caffeine on exercise performance in high ambient temperature. Eur J Appl Physiol, 2011. **111**(12): p. 3089-95.

20. Cohen, B.S., et al., Effects of caffeine ingestion on endurance racing in heat and humidity. Eur J Appl Physiol Occup Physiol, 1996. **73**(3-4): p. 358-63.

21. Begas, E., et al., In vivo evaluation of CYP1A2, CYP2A6, NAT-2 and xanthine oxidase activities in a Greek population sample by the RP-HPLC monitoring of caffeine metabolic ratios. Biomed Chromatogr, 2007. **21**(2): p. 190-200.

22. Ghotbi, R., et al., Comparisons of CYP1A2 genetic polymorphisms, enzyme activity and the genotype-phenotype relationship in Swedes and Koreans. Eur J Clin Pharmacol, 2007. **63**(6): p. 537-46.

23. Djordjevic, N., et al., Induction of CYP1A2 by heavy coffee consumption in Serbs and Swedes. Eur J Clin Pharmacol, 2008. **64**(4): p. 381-5.

24. Cornelis, M.C., et al., Coffee, CYP1A2 genotype, and risk of myocardial infarction. JAMA, 2006. **295**(10): p. 1135-41.

25. Palatini, P., et al., CYP1A2 genotype modifies the association between coffee intake and the risk of hypertension. J Hypertens, 2009. **27**(8): p. 1594-601.

26. Palatini, P., et al., Association of coffee consumption and CYP1A2 polymorphism with risk of impaired fasting glucose in hypertensive patients. Eur J Epidemiol, 2015. **30**(3): p. 209-17.

27. Salinero, J.J., et al., CYP1A2 Genotype Variations Do Not Modify the Benefits and Drawbacks of Caffeine during Exercise: A Pilot Study. Nutrients, 2017. **9**(3).

28. Algrain Haya A., T.R.M., Carrillo Andres E., Ryan Emily J., Kim Chul-Ho, Lettan Robert B. II, and Ryan Edward J., The Effects of a Polymorphism in the Cytochrome P450 CYP1A2 Gene on Performance Enhancement with Caffeine in Recreational Cyclists. Journal of Caffeine Research, 2016. **Volume 6**(Issue 1): p. 34-39.

29. Pataky, M.W., et al., Caffeine and 3-km cycling performance: Effects of mouth rinsing, genotype, and time of day. Scand J Med Sci Sports, 2016. **26**(6): p. 613-9.

30. Josse, A.R., et al., Associations between polymorphisms in the AHR and CYP1A1-CYP1A2 gene regions and habitual caffeine consumption. Am J Clin Nutr, 2012. **96**(3): p. 665-71.

31. Cohen, J., A power primer. Psychol Bull, 1992. **112**(1): p. 155-9.

32. Saunders, B., et al., Placebo in sports nutrition: a proof-of-principle study involving caffeine supplementation. Scand J Med Sci Sports, 2017; 27(11):1240-1247.

33. Killen, L.G., et al., Effects of caffeine on session ratings of perceived exertion. Eur J Appl Physiol, 2013. **113**(3): p. 721-7.

34. Green, J.M., et al., Caffeine influences cadence at lower but not higher intensity RPE-regulated cycling. Physiol Behav, 2017. **169**: p. 46-51.

35. Higgins, J.P. and K.M. Babu, Caffeine reduces myocardial blood flow during exercise. Am J Med, 2013. **126**(8): p. 730 e1-8.

36. Namdar, M., et al., Caffeine impairs myocardial blood flow response to physical exercise in patients with coronary artery disease as well as in age-matched controls. PLoS One, 2009. **4**(5): p. e5665.

37. Denden, S., et al., Gender and ethnicity modify the association between the CYP1A2 rs762551 polymorphism and habitual coffee intake: evidence from a meta-analysis. Genet Mol Res, 2016. **15**(2); doi: 10.4238/gmr.15027487.

38. Beedie, C.J., et al., Placebo effects of caffeine on cycling performance. Med Sci Sports Exerc, 2006. **38**(12): p. 2159-64.

39. Bchir, F., et al., Differences in pharmacokinetic and electroencephalographic responses to caffeine in sleep-sensitive and non-sensitive subjects. C R Biol, 2006. **329**(7): p. 512-9.

40. Silverman, K. and R.R. Griffiths, Low-dose caffeine discrimination and self-reported mood effects in normal volunteers. J Exp Anal Behav, 1992. **57**(1): p. 91-107.

Figure Captions

FIGURE 1—Mean (SEM) 10-km cycling times for all subjects (n = 101) under each caffeine treatment: 0, 2 and 4 mg/kg body mass.

*There was a significant decrease (p = 0.01) in 10-km cycling time during the 4 mg/kg caffeine trial compared to placebo.

p-values were generated from a model adjusted for visit

FIGURE 2—Average (mean ± SEM) 10-km cycling time by caffeine dose and *CYP1A2* genotype.

*2 mg/kg and **4 mg/kg caffeine trials significantly different from placebo (p^1 < 0.0001; p = 0.0005, respectively).

‡4 mg/kg caffeine trial significantly different (p = 0.02) from placebo.

[1]p-values were generated from models of individual genotypes and adjusted for visit

FIGURE 3— Change in 10-km cycling time to completion between genotypes (A) For group mean (SEM) between 2 mg/kg caffeine and placebo and (B) 4 mg/kg caffeine and placebo.

*CC genotype significantly different from AC (p = 0.002) and AA (p <0.0001).

FIGURE 4—10 km cycling times for AA, AC, CC genotypes (A) 2 mg/kg versus 0 mg/kg caffeine. Data points below the identity line indicate faster cycling times during 2 mg/kg versus 0 mg/kg caffeine dose. (B) 4 mg/kg versus 0 mg/kg caffeine. Data points below the identity line indicate faster cycling times during 4 mg/kg versus 0 mg/kg caffeine dose.

Figure 1

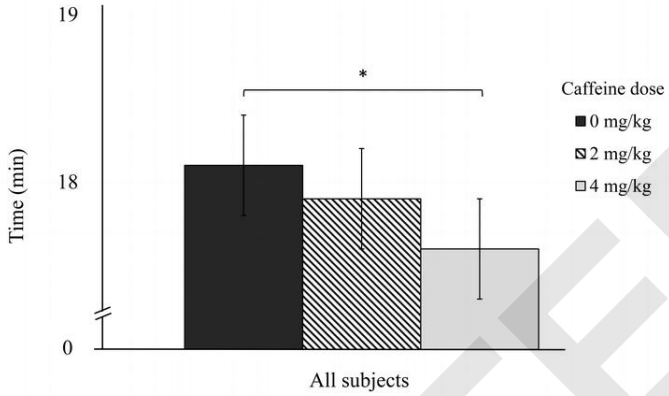

Figure 2

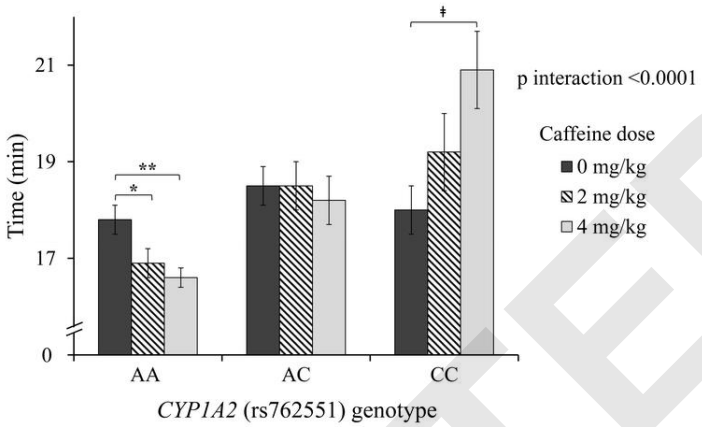

Figure 3

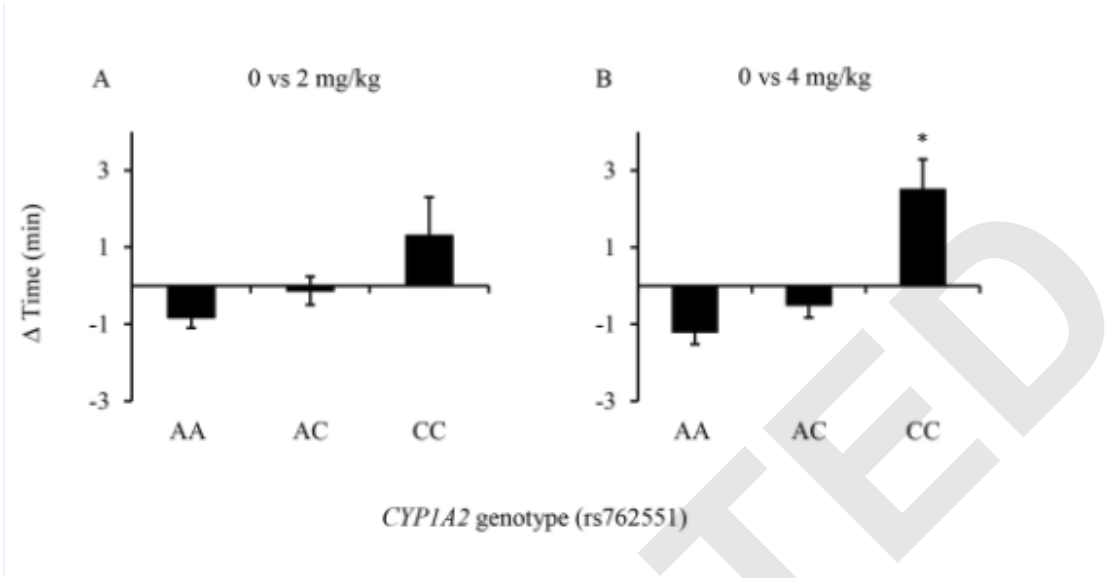

Figure 4

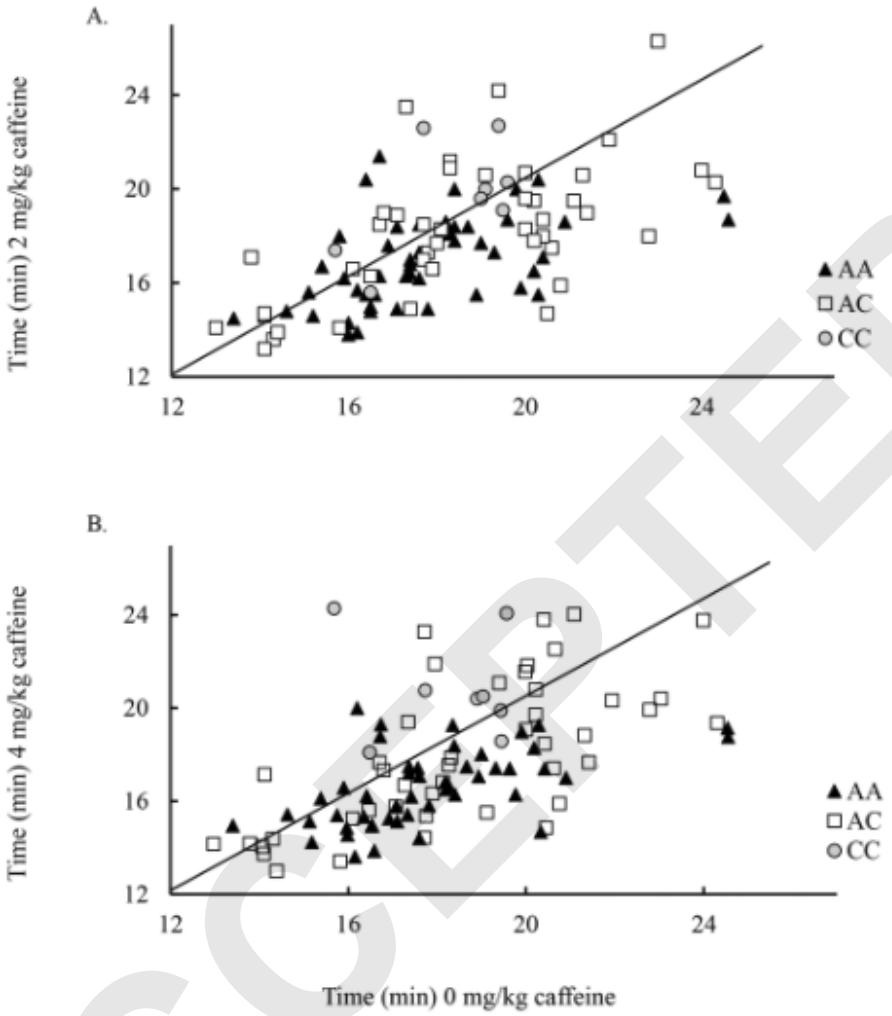

TABLE 1. Descriptive characteristics of participants by *CYP1A2* (rs762551) genotype

Characteristics	AA ($n = 49$)	AC ($n = 44$)	CC ($n = 8$)	p[1]
Height[2] (cm)	179 ± 7	177 ± 6	181 ± 10	0.15
Body mass (kg)	80.3 ± 12.2	79.7 ± 9.5	92.9 ± 24.9	0.07
Age (y)	24 ± 4	25 ± 5	25 ± 5	0.48
Body fat (%)	14.2 ± 4.4	13.8 ± 4.4	15.9 ± 6	0.49
VO_2peak ($L \cdot min^{-1}$)	3.9 ± 0.8	3.8 ± 0.7	3.9 ± 0.6	0.74
VO_2peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	49 ± 8	47 ± 12	44 ± 12	0.34
Caffeine Dietary[3] (mg per day)	87 ± 18	80 ± 20	38 ± 24	0.61
Caffeine Sport[4] (mg per day)	61 ± 13	89 ± 17	80 ± 74	0.49
Sport Type (%)				0.51
Endurance	46	49	5	
Power	45	45	10	
Mixed	62	25	13	

[1]p values were derived by using ANOVA, body mass variable was log transformed before analysis as it was not normally distributed, or for sport type by using Chi-Square.

[2]Mean ± SD (all values)

[3]Average dietary caffeine intake (excludes caffeine intake for sport)

[4]Average caffeine intake specifically for sport performance, i.e. training and competition (coffee, energy drinks, pre-workouts, gels, tablets etc.)

TABLE 2. TT time and caffeine dose by *CYP1A2* (rs762551) genotype with and without visit

			Caffeine dose (mg/kg)							
rs762551	Adj[1]	n[2]	0	2	4	R^2	p[3]	p[4]	p[5]	p[6]
AA	Yes	147	17.8	16.9	16.7	0.76	<0.0001	0.001	<0.0001	0.50
AA	No	147	17.8	17.0	16.6	0.70	<0.0001	0.007	<0.0001	0.50
AC	Yes	132	18.4	18.5	18.1	0.86	0.47	0.87	0.75	0.44
AC	No	132	18.6	18.4	18.0	0.74	0.37	0.95	0.37	0.55
CC	Yes	24	18.5	19.4	21.0	0.68	0.05	0.60	0.04	0.23
CC	No	24	18.3	19.6	20.8	0.56	0.06	0.37	0.05	0.45

[1]adjusted for visit

[2]number of visits = 3 x number of subjects in genotype

[3]overall p-value for comparison of three caffeine doses

[4,5,6]p-values for comparing time to completion between caffeine doses 0 and 2[4], 0 and 4[5], and 2 and 4 mg/kg[6]