Effects of caffeine on exercise performance of physically fit Thoroughbreds

Kathleen A. Savage, PhD; Patrick T. Colahan, DVM, PhD; Ian R. Tebbett, PhD; Brett L. Rice, BS; Lester L. Freshwater, MS; Christie A. Jackson, MS

Caffeine is a stimulant. As such, any concentration of caffeine or its metabolites in a postrace urine sample of a racehorse or Greyhound is considered a serious violation that can lead to severe penalties for the trainer in most pari-mutuel jurisdictions. Caffeine is considered by the Association of Racing Commissioners International (ARCI) to have no valid therapeutic use in racehorses; thus, when it is found in the blood or urine after a race, it is considered a class 2 violation with the corresponding ARCI penalty recommendations being suspension from racing for 6 months to 1 year, a fine of $1,500 to $2,500, and loss of any purse won during that race. However, because of the ubiquitous nature of caffeine in tea, coffee, sodas, and chocolate, trainers have argued for several years that low concentrations of caffeine in racing animals are inconsequential to racing performance and are associated with inadvertent environmental contamination.

Caffeine is extensively metabolized in the liver by the cytochrome P450 system. Its major metabolites are theobromine, theophylline, and paraxanthine, which are produced by demethylation of caffeine at positions 1, 7, and 3, respectively. The major pharmacologic actions of caffeine are stimulation of the CNS (including respiratory stimulation), diuresis, stimulation of cardiac muscle, and relaxation of smooth muscle, especially bronchial muscle. These effects are induced partly by inhibition of phosphodiesterase, thus causing effects similar to those of β-adrenoceptor agonists, and partly by antagonism at purine receptors (A1- and A2-receptor subtypes).

Thus, it follows that caffeine has substantial potential for use as a performance-altering drug. In humans, caffeine can improve physical performance and endurance during prolonged activity of submaximal intensity. This was initially documented in a study in which investigators evaluated the effects of 330 mg of caffeine given to trained cyclists 1 hour before they performed exercise in which they cycled at 80% of maximal oxygen consumption ($\dot{V}O_{2max}$) until they reached exhaustion. Performance was improved in those cyclists (75 minutes to reach exhaustion after placebo treatment vs 96 minutes to reach exhaustion after caffeine treatment).

Other studies on the effects of caffeine in which investigators have provided better control for confounding factors and selected better performance assessments to simulate competitive conditions have been conducted. The collective outcome of those studies was that endurance performance could be improved by 20% to 50% when elite and recreationally trained human athletes ingested caffeine (dosage range, 3 to 13 mg/kg). This prolongation of the time until exhaustion during endurance exercise is believed to be a result of increased lipolysis that leads to a glycogen-sparing effect. It is interesting that dosages of 3, 5, and 6 mg/kg consistently enhanced endurance performance but resulted in urinary concentrations below the acceptable limit set by the International Olympic Committee (IOC). Currently for humans, the IOC stipulates that urine caffeine concentrations ≥ 12 µg/mL are considered a violation of their rules. Caffeine dosages of < 9 mg/kg will generally result in urinary caffeine concentrations below the violative limit.

Recently, there has been interest in determining the effects of caffeine on short bouts of intense exercise (approx 100% $\dot{V}O_{2max}$ that lasted approx 5 minutes), but reports on caffeine's ergogenic activity during this type of exercise are conflicting. One group report-
ed a nonsignificant increase in the time for cyclists to reach exhaustion when performing at 100% VO2max after 250 mg of caffeine, compared with the time to reach exhaustion when given a placebo. In another study, subjects drank coffee or a placebo solution, then ran at a predetermined pace for a distance of 1,100 m, and then ran a distance of 400 m as fast as possible. In that study, the mean speed for the final 400 m was faster and mean oxygen consumption (VO2) during this period of exercise was higher for the group that drank coffee. The ergogenic effect of caffeine during short-term intense exercise is not reportedly associated with a glycogen-sparing effect, which suggests that performance enhancement may be attributable to a direct action on muscles or altered function of the CNS.

Effects of caffeine have been studied but to a slightly lesser degree for sprint performance in humans. Caffeine ingestion did not have an effect on power output or muscular endurance during short maximal bouts of cycling. However, a study conducted on the effects of 250 mg of caffeine on trained swimmers revealed that caffeine resulted in a 2% to 4% increase in velocity, although performance times were not reported. Thus, because of a lack of reliable studies in this area, it has been difficult to determine the effects, if any, of caffeine on periods of brief, intense exercise.

In light of this information and because of the vast improvements in analytic methods during recent years, regulatory agencies are under increasing pressure to consider threshold concentrations for caffeine in animal athletes. However, there is a paucity of information on this. In 1 study, investigators evaluated the effects of various doses of caffeine on locomotor activity of horses and concluded that the highest dose that caused no effect was 2 mg/kg, IV. They suggested that no-effect thresholds for horses should be set at a maximum concentration of 2 μg of caffeine/mL of plasma or 5 μg of caffeine/mL of urine. However, that study was performed on horses at rest and did not take into account any of the potential physiologic and biochemical effects of caffeine on exercise performance. Therefore, the study reported here was designed to correlate exercise performance (physiologic and biochemical effects) of physically fit Thoroughbreds with dose of caffeine administered and urinary concentrations of caffeine.

Materials and Methods

Animals—Ten Thoroughbreds (6 geldings, 2 stallions, and 2 mares) between 3 and 11 years of age were used in the study. We determined that the horses were orthopedically sound and did not have detectable diseases of the respiratory tract and cardiovascular system. Horses were housed in a small paddock, fed 2 kg of commercial concentrate twice daily, and provided ad libitum access to hay and water. All horses received preventive medical procedures, including medications for control of internal parasites and vaccinations against appropriate infectious diseases. General health of horses was monitored on a daily basis. All horses underwent a period of training on a high-speed treadmill (3 to 5 d/wk) for at least 8 weeks prior to the onset of the study. The methods used in this study were approved by the University of Florida Institutional Animal Care and Use Committee.

Experimental procedure—Horses were randomly assigned to initially receive caffeine or a control solution. Each horse received both treatments in a crossover design with a 3-week interval between treatments. The investigators were aware of the treatment administered to each horse.

Exercise training was continued during the study, with 2 days of rest after a horse performed an exercise stress test. Experimental days were days on which each horse performed an exercise stress test and measurements were obtained. On those days, each horse was weighed prior to administration of caffeine or the control solution. In addition, mean ± SD values for environmental conditions (ambient temperature, relative humidity, and barometric pressure) were recorded.

Anhydrous caffeine (0.9% NaCl) solution containing sodium benzoate to achieve a final concentration of 50 mg/mL. Caffeine was administered IV at a dosage of 2.5 mg/kg. This dosage was determined from preliminary clearance studies conducted by personnel at our institution. An equal volume of heparinized saline solution was administered as the control solution.

One hour after administration of caffeine or the control solution, a horse was walked onto a high-speed treadmill and an open-flow indirect-calorimeter face mask was positioned over the horse’s nose and mouth. The open-flow calorimeter contained oxygen and carbon dioxide sensors; these were calibrated in accordance with the manufacturer’s instructions by use of certified primary standard gas mixtures prior to each exercise test. A baseline heart rate was then recorded.

Incremental exercise testing was performed on each horse to determine peak VO2 (VO2peak). Exercise was initiated on a flat treadmill at a speed of 4 m/s for 2 minutes (defined as the warm-up period). The slope of the treadmill was then increased to 6° (10%) and speed increased to 8 m/s for 1 minute. Speed of the treadmill was then increased by 1 m/s for each successive minute until the onset of fatigue. Fatigue was defined as the point at which the horse failed to maintain its position on the treadmill despite humane encouragement from the investigators. Time to fatigue and speed of the treadmill at fatigue were recorded for each horse.

Oxygen consumption was measured continuously and recorded at 10-second intervals. Oxygen consumption at time of fatigue was defined as VO2peak. Heart rate was measured from time-synchronized ECG recordings. Caffeine was administered as the control solution. A horse was walked onto a high-speed treadmill and an open-flow indirect-calorimeter face mask was positioned over the horse’s nose and mouth. The open-flow calorimeter contained oxygen and carbon dioxide sensors; these were calibrated in accordance with the manufacturer’s instructions by use of certified primary standard gas mixtures prior to each exercise test. A baseline heart rate was then recorded.

Venous blood samples were collected from each horse before administration of caffeine or the control solution and immediately before and after each exercise test. Samples were used for determination of lactate and caffeine concentrations. Blood samples were collected into heparin-containing tubes and immediately placed in crushed ice. Samples were centrifuged in a refrigerated centrifuge and plasma harvested within 60 minutes after collection. Lactate concentrations were measured within 24 hours after sample collection.

A urine sample was collected from each horse 1 hour after administration of caffeine or control solution. Urine samples were used for determination of the urinary caffeine concentration at the time of exercise. A high-performance liquid chromatography method that has been extensively validated by the authors was used to determine concentrations of the analytes.

Statistical analysis—A mixed-effects linear model was used to compare the various variables measured between the treatments. A mixed-effect linear model was fit by use of generalizing least squares to explicitly model the correlation structure between the repeated measures on each horse. All analyses were conducted by use of a statistical program.
Treatment was defined as caffeine or control solution. Period was defined as the interval in which a horse received 1 of the 2 treatments, and time was defined as a time point within a period at which a measurement was obtained. To reduce the possibility that random variation may have caused a spurious finding of significance because of the large number of variables analyzed, significance was set at $P \leq 0.01$.

**Results**

**Measures of performance**—Respiratory measures of performance obtained during the experiments were summarized (Table 1). Mean ± SD $\dot{V}O_2$ increased from 6.76 ± 2.99 mL/kg/min at warm up to 139.26 ± 13.49 mL/kg/min at fatigue when horses administered the control solution and from 7.37 ± 1.69 to 131.99 ± 10.70 mL/kg/min for horses when administered caffeine. Mean production of carbon dioxide increased from 7.37 ± 2.86 mL/kg/min at warm up to 157.17 ± 23.92 mL/kg/min at fatigue when horses were administered the control solution and from 7.2 ± 1.69 to 134.1 ± 40.6 mL/kg/min when horses were administered caffeine. Mean production of carbon dioxide increased from 7.37 ± 2.86 mL/kg/min at warm up to 157.17 ± 23.92 mL/kg/min at fatigue when horses were administered the control solution and from 7.2 ± 1.69 to 134.1 ± 40.6 mL/kg/min when horses were administered caffeine. Mean heart rate also increased from 54.9 ± 16.0 beats/min at baseline to 206.0 ± 18.0 beats/min at fatigue when horses were administered the control solution and from 61.5 ± 17.2 beats/min at baseline to 211.5 ± 11.9 beats/min at fatigue when horses were administered caffeine. Oxygen pulse, which provides an indication of the functioning of the entire cardiopulmonary system, increased from 0.07 ± 0.03 mL/kg/beat at warm up to 0.68 ± 0.07 mL/kg/beat at fatigue when horses were administered the control solution and from 0.07 ± 0.01 mL/kg/beat at warm up to 0.62 ± 0.06 mL/kg/beat at fatigue when horses were administered caffeine.

Mean lactate concentration increased from 7.1 ± 1.9 mg/dL before exercise to 136.8 ± 17.5 mg/dL after exercise when horses were administered the control solution and from 7.2 ± 2.3 to 134.1 ± 40.6 mg/dL when horses were administered caffeine. Mean time to fatigue was 272 ± 34 seconds when horses were administered the control solution and 258 ± 26 seconds when horses were administered caffeine.

**Concentrations of caffeine and metabolites**—Concentrations of caffeine and its metabolites were determined in serum and urine samples obtained at specific time points during the experiments. Mean concentration of caffeine at the time of exercise was 5.8 µg/mL in urine and 3.3 µg/mL in serum.

**Discussion**

Caffeine stimulates the respiratory center. Therefore, we continuously monitored $\dot{V}O_2$, carbon dioxide production, and heart rate during the period of exercise. These measures increased as expected for fit Thoroughbreds during the period of exercise until the horses reached a point of fatigue, and no significant differences were detected between horses when they were administered the control solution or caffeine. Lactate concentrations were measured immediately before and after each exercise test. Our results were in concurrence with the expected baseline and postexercise concentrations, with no significant differences

<table>
<thead>
<tr>
<th>Speed (m/s)</th>
<th>Treatment</th>
<th>Heart rate (beats/min)</th>
<th>$\dot{V}O_2$ (mL/kg/min)</th>
<th>Oxygen pulse* (mL/kg/beat)</th>
<th>$\dot{V}CO_2$ (mL/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Control</td>
<td>54.9 ± 16.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>56.5 ± 17.2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>104.6 ± 14.6</td>
<td>6.76 ± 2.99</td>
<td>0.07 ± 0.03</td>
<td>7.37 ± 2.86</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>108.0 ± 12.0</td>
<td>7.33 ± 1.69</td>
<td>0.07 ± 0.01</td>
<td>7.97 ± 2.11</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>144.9 ± 15.7</td>
<td>25.61 ± 3.96</td>
<td>0.18 ± 0.02</td>
<td>24.17 ± 4.11</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>145.8 ± 26.7</td>
<td>28.04 ± 7.57</td>
<td>0.20 ± 0.05</td>
<td>26.42 ± 6.10</td>
</tr>
<tr>
<td>9</td>
<td>Control</td>
<td>195.4 ± 5.9</td>
<td>96.31 ± 23.20</td>
<td>0.51 ± 0.13</td>
<td>81.35 ± 16.85</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>192.8 ± 17.7</td>
<td>93.61 ± 16.92</td>
<td>0.48 ± 0.09</td>
<td>82.00 ± 19.68</td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>199.7 ± 2.9</td>
<td>118.67 ± 9.27</td>
<td>0.59 ± 0.05</td>
<td>111.85 ± 20.18</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>201.8 ± 8.4</td>
<td>118.10 ± 13.04</td>
<td>0.58 ± 0.07</td>
<td>113.97 ± 21.47</td>
</tr>
<tr>
<td>11</td>
<td>Control</td>
<td>205.7 ± 2.9</td>
<td>128.7 ± 9.52</td>
<td>0.62 ± 0.05</td>
<td>130.85 ± 22.67</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>210.8 ± 8.7</td>
<td>125.39 ± 11.37</td>
<td>0.60 ± 0.06</td>
<td>132.16 ± 22.74</td>
</tr>
<tr>
<td>12</td>
<td>Control</td>
<td>211.0 ± 4.5</td>
<td>137.34 ± 7.33</td>
<td>0.64 ± 0.04</td>
<td>146 ± 22.02</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>219.8 ± 6.8</td>
<td>125.77 ± 10.54</td>
<td>0.57 ± 0.06</td>
<td>137.32 ± 21.63</td>
</tr>
<tr>
<td>13†</td>
<td>Control</td>
<td>228.0</td>
<td>144.48</td>
<td>0.63</td>
<td>163.74</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>234.0</td>
<td>124.75</td>
<td>0.53</td>
<td>132.88</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Control</td>
<td>206.6 ± 18.0</td>
<td>139.26 ± 13.49</td>
<td>0.68 ± 0.07</td>
<td>157.17 ± 22.92</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>211.5 ± 11.9</td>
<td>131.99 ± 10.70</td>
<td>0.62 ± 0.06</td>
<td>151.01 ± 22.27</td>
</tr>
</tbody>
</table>

---

*Oxygen pulse was calculated as peak oxygen consumption ($\dot{V}O_2$) divided by heart rate; peak $\dot{V}O_2$ was defined as the $\dot{V}O_2$ at time of fatigue. †Only 1 horse reached this speed.

$\dot{V}O_2 = \text{Volume of carbon dioxide produced. ND = Not determined.}$
being found between horses when they were administered the control solution or caffeine. Finally, the time to fatigue for each horse was monitored, and there were no significant differences in time to fatigue between horses when they were administered the control solution or caffeine.

A mixed-effects linear model was used to analyze these data. Use of this technique explicitly models the correlation structure between the repeated measures for each horse and thus provides an extremely sensitive statistical analysis.

Concentrations of caffeine and its major metabolites were measured in serum and urine for the purpose of correlating the concentration of caffeine found at the time of exercise (1 hour after administration) with any effects, or lack thereof, of caffeine on exercise performance. Mean concentration of caffeine at the time of exercise was 5.8 µg/mL in urine and 3.3 µg/mL in serum.

Many states adhere to a zero-tolerance policy for most drugs in racing animals. Thus, any amount of caffeine were found in the blood or urine of a horse after a race, the trainer would face penalties recommended for a class 2 violation of the rules of racing, as described previously. Numerous jurisdictions have considered it to be a positive result for caffeine when the estimated concentration is as low as 20 ng/mL of serum.

Findings of the study reported here are in close agreement with those of another study. However, investigators in that study measured natural locomotor activity, which has not been proven to correlate with physiologic or biochemical responses to exercise. At best, that study provides a good starting point from which experiments can be designed to examine the effects of similar doses of caffeine during a period of exercise.

In another study, investigators argued that measurements obtained during incremental exercise procedures, such as the method used in the study reported here, correlate well with aerobic and anaerobic capacities in horses. They believed that such protocols may be an appropriate method to use in determining the effects of drugs on athletic performance. However, we did not detect a positive effect of caffeine on the physiologic variables measured in horses by use of an incremental treadmill exercise protocol. Similarly, the statistical power of the study reported here could not detect extremely small changes associated with the amount of caffeine administered. Analysis of our results does reveal that caffeine administered at this dosage did not exert a large effect on these variables.

Although urine testing is adequate and acceptable in jurisdictions with a zero-tolerance policy, it is doubtful whether urinary concentrations can be correlated with serum concentrations or pharmacologic effects. Urinary concentrations of a drug represent only what is being eliminated from an animal and not the circulating concentration of the drug that reaches target organs and tissues. Thus, it may be beneficial to the horse-racing industry to consider implementing testing of serum samples rather than urine samples.

The study reported here investigated the effects of caffeine on fit Thoroughbreds. A treadmill was used, which allowed us to obtain physiologic and biochemical measurements and correlate them with other measures of performance, such as time to fatigue and serum and urinary concentrations of caffeine and its metabolites in horses performing intense physical exercise. We believed this experimental method provided a more accurate and realistic determination of the effects of caffeine on performance than has been reported in any other study conducted on this drug. By use of this method, there were no significant differences for any of the variables measured during the exercise period between horses when they were administered the control solution or when they were administered caffeine.

References