Effect of caffeine on metabolism, exercise endurance, and catecholamine responses after withdrawal

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Van Soeren, M. H., and T. E. Graham. Effect of caffeine on metabolism, exercise endurance, and catecholamine responses after withdrawal. J. Appl. Physiol. 85(4): 1493–1501, 1998.—In this study the effects of acute caffeine ingestion on exercise performance, hormonal (epinephrine, norepinephrine, insulin), and metabolic (free fatty acids, glycerol, glucose, lactate, expired gases) parameters during short-term withdrawal from dietary caffeine were investigated. Recreational athletes who were habitual caffeine users (n = 6) (maximum oxygen uptake 54.5 ± 3.3 ml·kg⁻¹·min⁻¹ and daily caffeine intake 761.3 ± 11.8 mg/day) were tested under conditions of no withdrawal and 2- and 4-day withdrawal from dietary caffeine. There were seven trials in total with a minimum of 10 days between trials. On the day of the exercise trial, subjects ingested either dextrose placebo or 6 mg/kg caffeine in capsule form 1 h before cycle ergometry to exhaustion at 80–85% of maximum oxygen uptake. Test substances were assigned in a random, double-blind manner. A final placebo control trial completed the experiment. There was no significant difference in any measured parameters among days of withdrawal after ingestion of placebo. At exhaustion in the 2- and 4-day withdrawal trials, there were significant increases in plasma norepinephrine in response to caffeine ingestion. Caffeine-induced increases in serum free fatty acids occurred after 4 days and only at rest. Subjects responded to caffeine with increases in plasma epinephrine (P < 0.05) at exhaustion and prolonged exercise time in all caffeine trials compared with placebo, regardless of withdrawal from caffeine. It is concluded that increased endurance is unrelated to hormonal or metabolic changes and that it is not related to prior caffeine habituation in recreational athletes.

ACUTE INGESTION OF CAFFEINE before exercise has been shown to prolong exercise endurance, increase plasma epinephrine and serum free fatty acids (FFAs), and spare muscle glycogen (14, 18, 29). Research has been directed toward establishing a causal relationship between these parameters on the basis of the hypothesis that caffeine ingestion resulted in a rise in circulating catecholamines, which mobilized FFAs from adipocytes, thus increasing the amount of fat available for active muscle (10, 14, 22). The ergogenic effect was thought to be a result of utilization of fat and a resulting sparing of muscle glycogen (14). These concepts were tested into the 1990s (18, 29), but more recent research has failed to support this hypothesis (7, 19).

In humans at rest, Robertson et al. (27) found that moderate prolonged caffeine use was sufficient to dampen the caffeine-induced epinephrine response. There are few exercise studies (2, 21, 32) that test the assumptions that prior caffeine use alters hormonal and metabolic responses to acute caffeine ingestion and that this process is reversible. If epinephrine is a critical element in the ergogenic effect after caffeine consumption, then attenuation of caffeine-induced increases in plasma epinephrine in habitual caffeine consumers may also dampen the performance and metabolic effects of caffeine during exercise.

In vivo, the major mechanism through which caffeine acts is as an adenosine-receptor antagonist (1), and all tissues with adenosine receptors may be affected by caffeine exposure. Chronic exposure to caffeine results in increases in both available adenosine receptors and/or receptor-mediated activity in humans and animals (4, 8). Thus, during periods of short-term withdrawal from dietary caffeine, acute caffeine ingestion may result in an enhancement of the caffeine-induced responses, such as increases in plasma epinephrine, during exercise. We (32) previously demonstrated that increased epinephrine responses occur during submaximal exercise in habitual caffeine users with or without acute caffeine ingestion. Others have reported conflicting epinephrine responses in subjects whose prior caffeine usage was known (2, 18, 30). A single study has specifically addressed the issue of habituation to caffeine during exercise and found no change in substrate utilization after a 48-h withdrawal from caffeine (21). However, these authors did not measure methylxanthines, catecholamines, or endurance, all of which may be critically altered after withdrawal from dietary caffeine.

The present study was designed to test the hypothesis that caffeine-induced changes in metabolic and catecholamine responses and in exercise performance are less pronounced during periods of chronic caffeine exposure. Chronic caffeine exposure may influence the ergogenic response to caffeine ingestion. It is anticipated that this dampening of responses will be reversed after short-term withdrawal from caffeine. Recreational athletes were tested during exercise to exhaustion under conditions of no withdrawal and 2 and 4 days of withdrawal from all dietary caffeine. Caffeine-induced hormonal and metabolic responses and exercise endurance were examined to determine the effect of habituation and withdrawal in caffeine users during high-intensity cycling exercise under conditions of placebo or acute caffeine ingestion (6 mg/kg).

METHODS

Subjects

Six recreational male athletes agreed to participate in the study after being informed of the nature of the experiments. Subjects were nonsmokers and had been actively cycling...
and/or running five times per week for at least 1 yr (n = 5) or five times per week for 3 mo (n = 1). Subjects were 36.7 ± 4.2 (SE) yr of age, weighed 75.1 ± 1.6 kg, and had an average daily caffeine consumption from all sources equivalent to 761.3 ± 11.8 mg/day (based on self-reporting and using amounts recommended by Conlee (9)). Their primary source of dietary caffeine was from coffee. Each subject signed a consent form that outlined possible risks of the procedure. The protocol was approved by the University of Guelph Ethics Committee.

Experimental Procedure

Subjects reported to the laboratory before the start of the experiment for an incremental maximum oxygen uptake \((\dot{V}O_2max)\) test on a cycle ergometer from which a power output required to elicit 80–85% \(\dot{V}O_2max\) was calculated. Mean \(\dot{V}O_2max\) was 54.5 ± 3.3 ml · kg\(^{-1}\) · min\(^{-1}\). They returned for at least one additional trial ride of 30-min duration to confirm the power output required to elicit 80–85% \(\dot{V}O_2max\) and to habituate themselves to the protocol.

Subjects were requested to maintain similar exercise patterns throughout the duration of the experiments, to follow constant activity patterns for the 3 days before each test, and to abstain from exercise 24 h before each trial. They were requested to consume similar diets for 48 h before each experiment, and each maintained a diet record for this period. When they were required to abstain from caffeine (i.e., withdrawal), they were cautioned to avoid sources of methylxanthines such as tea, coffee, chocolate, cola drinks, and caffeine-containing over-the-counter pharmaceuticals, and they were asked to report any deviations from these instructions. For the no-withdrawal trials they were allowed to consume products containing caffeine up to the morning of the trial. Subjects were requested to consume a small meal 2–4 h before the experiment, which was identical for each trial. Dietary consistency was confirmed through self-reported diet records and questioning before each trial. Subjective withdrawal symptoms were recorded for each subject before each exercise trial.

Seven exercise trials to exhaustion were completed by all subjects. A minimum of 10 days between all trials was established to minimize any possible effects of the abstinence from normal caffeine intake. The first two trials were completed without any withdrawal from dietary caffeine, and subjects were assigned randomly either dextrose placebo (Pl 0d) or caffeine (Caf 0d; 6 mg/kg body wt; Caffeine US Pharmaceutical/Federal Commerce Commission catalog no. C-0905, A&C American Chemicals). It was believed that the tests under normal habituation would most likely mimic the responses in other comparable studies, and therefore the protocol was designed with these tests before withdrawal was commenced. Subsequent exercise trials were conducted after periods of either a 2- or 4-day withdrawal from dietary caffeine and are designated as Pl 2d and Pl 4d, for the placebo 2- and 4-day withdrawals, respectively, and as Caf 2d and Caf 4d for the caffeine 2- and 4-day withdrawals, respectively. Both the sequence of days of withdrawal and the receipt of caffeine or placebo were assigned in a random, double-blind manner for all trials. After these experiments all subjects participated in a placebo-repeat trial (Pl R) (administered in a single-blind manner) without any period of withdrawal from caffeine. The protocol was identical to the previous trials, except that drug was used in a holdover study rather than in a withdrawal study. After these experiments all subjects participated in a placebo-repeat trial (Pl R) (administered in a single-blind manner) without any period of withdrawal from caffeine. The control was designed to test for a training effect after the 3- to 6-mo period required to complete the trials. At the completion of each exercise trial, subjects were asked to complete a questionnaire inquiring whether they could determine which drug they had received and to describe the basis on which they made this choice.

Experimental Protocol

The protocol for each trial was identical. Trials were held at the same time of day for each subject and typically started at 0900. Before each trial, a venous catheter was inserted into the antecubital vein and was kept patent with a saline infusion. The subject then rested in a supine position for 15 min before any further testing. A resting blood sample of 12 ml was then collected, and the subject ingested the trial substance in gelatin capsules with water (– 60 min). After 1 h of rest, during which time the subject was allowed to sit or lie quietly, another resting blood sample was obtained (0 min). Subjects then exercised to exhaustion at 80–85% \(\dot{V}O_2max\) on a cycle ergometer. Each subject completed a 5 min warm-up at a lower power output, which was identical for all trials and was not considered as part of the total exercise time.

Samples for expired air were taken every 5 min during the initial 20 min of exercise, and blood samples (12 ml) were taken at 5 and 20 min after the warm-up period. A final blood and an expired air sample were taken within 2 min before the point of exhaustion. No samples were taken between 20 min and exhaustion so that subjects could not count samples as a crude timing device. Exhaustion was determined by the investigator when cadence could no longer be maintained at a rate of 90% of the subject's set rate. When the researchers thought the subject was close to exhaustion, a sample was taken. If the subject did not demonstrate decreased cadence within 2 min of that sample, another sample was taken, and the former was discarded. Water was given ad libitum during exercise. Subjects did not have access to any indication of time after the initial 20-min sampling period during the exercise.

Analyses

Expired gas samples were analyzed for fractions of \(O_2\) and \(CO_2\) with an Applied Electrochemical S-3A \(O_2\) analyzer and Sensor Medics LB-2 \(CO_2\) detector, respectively. Expired volume was determined with a Parkinson-Cowan volumeter. The analyzers were calibrated with gases of known concentrations, previously determined by micro-Scholander technique. The volume meter was calibrated with a Tissot spirometer. Blood samples were immediately separated into two aliquots. One was transferred immediately into a test tube for serum, and 7 ml were transferred into a sodium heparinized tube. Hematocrit was immediately measured in triplicate from the latter tube by using high-speed centrifugation. Hemoconcentration occurred in all the exercise samples, but there was no difference between trials. An aliquot of 100 µl heparinized blood was added to 500 µl of 0.3 M perchloric acid. A solution containing 120 µl of 0.24 M EGTA and reduced glutathione was then added to the remaining heparinized whole blood. The EGTA and glutathione treated plasma was analyzed for epinephrine and norepinephrine (NE) concentration by using HPLC (Waters) as described by Welker et al. (34). Plasma caffeine was analyzed by using fully automated HPLC (Waters). For the latter procedure, plasma samples were subjected to centrifugal filtration by using Ultrafree-MC polysulfone filter units (Millipore UFC37GC). Filtered plasma and the internal standard 6-hydroxyethyltheophylline were injected onto a resolve Radial-Pak cartridge (Waters 84624) by using a Radial Compression Module 8 × 10 pressure module. Caffeine was measured at 254-nm wavelength and sensitivity range of 0.01 absorbance units full scale. Reagents for standards were obtained from Sigma Chemical.

The whole-blood acid extracts were analyzed enzymatically in triplicate for lactate and glucose as described by Bergmeyer (3). Serum was analyzed enzymatically in triplicate for
FFAs (26) and glycerol (16). Plasma insulin was analyzed by using $^{125}$I radioimmunossay kit (Diagnostic Products Code-a-Count Insulin kit). Diet analysis was completed as described by Dibbee and Graham (12).

Statistics

Statistical analysis of respiratory, exercise, and blood data was conducted by using repeated-measures analysis of variance for drug and withdrawal. Bonferroni corrected $t$ tests were used to adjust $\alpha$ levels to limit the possibility of a type I error. A one-way analysis of variance was used to test the difference between Pl 0d and Pl R data as well as Caf 0d and Pl R data. Experimental significance is described as $P < 0.05$, and all data are reported as means ± SE. Because of the small number of subjects, observed power calculations were completed and were found to be ~0.500 for all nonsignificant data.

RESULTS

There was no significant difference between the Pl 0d and the Pl R trials in any of the measured parameters. Although the original purpose of the Pl R trial was for use as a comparison with the Pl 0d trial, statistical comparison of the Pl R and Caf 0d data was completed. These data followed a similar pattern of significance as did the Pl 0d vs. Caf 0d data, with the exception of the time data. Therefore, the Pl R data will be shown only with respect to time to exhaustion (Fig. 1).

Time

Time to exhaustion was significantly increased over placebo in all caffeine trials regardless of the period of withdrawal (Fig. 1). Ingestion of caffeine significantly increased the time to exhaustion in all subjects after no withdrawal (74.8 ± 8.8 vs. 59.0 ± 3.7 min for caffeine vs. placebo, respectively), the 2-day withdrawal (81.1 ± 6.7 vs. 59.5 ± 8.4 min), and the 4-day withdrawal (81.5 ± 6.4 vs. 63.6 ± 5.6 min). In contrast to the Pl 0d trial, the time to exhaustion in the Pl R trial was not significantly different than in the Caf 0d trial. This increase in time for the Pl R trial is heavily influenced by a single subject whose time increased from 70 to 124 min (Pl 0d vs. Pl R, respectively). When this subject’s data are removed from the analyses, the Pl R and Caf 0d trials are significantly different, because all other subjects performed similarly in both Pl trials.

Catecholamines

Ingestion of caffeine resulted in a significant increase in plasma epinephrine concentration in all trials at exhaustion compared with that of placebo regardless of the days of withdrawal ($F = 11.090, P = 0.007$; Fig. 2). There was an effect of withdrawal on NE responses to caffeine. Caffeine ingestion resulted in significantly elevated plasma NE concentrations over the placebo conditions at exhaustion in the 2- and 4-day withdrawal trials, but NE levels were similar to placebo in the no-withdrawal trial ($F = 8.793, P = 0.006$; Fig. 3).

Insulin

Insulin concentration declined with exercise, and there were no significant differences in any of the trials after ingestion of caffeine (data not shown). Initial values for the no-withdrawal trials were 9.0 ± 3.0 and 10.6 ± 3.6 µU/ml at −60 min, which decreased to 2.7 ± 0.4 and 5.5 ± 2.4 µU/ml at exhaustion, for placebo and caffeine, respectively.

Metabolic Data

FFAs. There is a significant effect of caffeine ingestion on FFA concentrations ($F = 25.100, P = 0.004$). This significant increase occurred in the 4-day trial after caffeine ingestion when FFA concentration increased at rest from 0.22 ± 0.04 mM (−60 min) before caffeine ingestion to 0.45 ± 0.12 mM (0 min) compared with the Pl 4d data. At no other time or treatment were FFA concentrations significantly altered by caffeine or withdrawal (Table 1).

Glycerol. Regardless of whether the treatment was placebo or caffeine, serum glycerol concentration increased gradually during the initial exercise period and was elevated at exhaustion in all trials ($F = 46.219, P = 0.0001$) (Table 1). After the 4-day withdrawal from caffeine ingestion, there was an increase in glycerol concentration compared with that in the placebo trial, but this was not significant.

Glucose. Blood glucose concentration was not altered by either days of withdrawal or caffeine ingestion (Table 2). A low initial glucose concentration at −60 min in the Caf 0d trial was demonstrated in four of the six subjects despite their reported similar diet and activity pattern before experimentation.

Lactate. Blood lactate increased in response to exercise in all trials (Fig. 4). After the 2 days of withdrawal, acute ingestion of caffeine resulted in a tendency toward an increase in lactate concentration at exhaus-
amounts of residual caffeine may be detected in plasma. When there was no withdrawal, the initial mean plasma caffeine concentration was 7.11 ± 2.26 (caffeine) and 10.99 ± 3.09 (placebo) µM. In the Pl 0d trial, the caffeine concentration was significantly higher than in other placebo trials. Despite the higher initial value in the Caf 0d trial, there was no significant difference in maximal concentrations measured in the caffeine trials.

Expired gases. There was no significant difference found in any of the measured parameters of oxygen

Fig. 2. Plasma epinephrine concentration during exercise under conditions of no withdrawal (A) and 2-day (B) and 4-day withdrawal (C) from dietary caffeine. Lines are mean data, and vertical bars are SE; n = 6 subjects. Time indicates sampling time: −60, preingestion; 0, 1 h of rest postingestion at start of exercise; 5 and 20, time from start of exercise; exhaustion (exh), end of exercise when subject could no longer maintain a power output of 85% maximum oxygen uptake. ■, Placebo; ○, caffeine. *Significant difference from placebo, P < 0.05.

Fig. 3. Plasma norepinephrine concentrations during exercise under conditions of no withdrawal (A) and 2-day (B) and 4-day withdrawal from dietary caffeine (C). Lines are mean data, and vertical bars are SE; n = 6 subjects. ■, Placebo; ○, caffeine. Organization is as in Fig. 2. *Significant difference from placebo, P < 0.05.
Table 1. Serum free fatty acids and glycerol

<table>
<thead>
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<th>Treatment</th>
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<th>FFA</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-60</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Pl 0d</td>
<td>0.27±0.06</td>
<td>0.28±0.08</td>
<td>0.28±0.07</td>
</tr>
<tr>
<td>Pl 2d</td>
<td>0.19±0.03</td>
<td>0.15±0.03</td>
<td>0.22±0.05</td>
</tr>
<tr>
<td>Pl 4d</td>
<td>0.17±0.02</td>
<td>0.26±0.07</td>
<td>0.20±0.03</td>
</tr>
<tr>
<td>Caf 0d</td>
<td>0.22±0.03</td>
<td>0.33±0.04</td>
<td>0.24±0.04</td>
</tr>
<tr>
<td>Caf 2d</td>
<td>0.25±0.05</td>
<td>0.26±0.08</td>
<td>0.23±0.06</td>
</tr>
<tr>
<td>Caf 4d</td>
<td>0.22±0.04</td>
<td>0.45±0.12*</td>
<td>0.25±0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE in mM for 6 subjects for placebo (Pl) and caffeine (Caf) trials; 0d, no withdrawal; 2d, 2-day withdrawal; 4d, 4-day withdrawal. Time points indicate before 4d ingestion (−60), after 1 h rest (0), after 5 and 20 min of exercise, and at exhaustion (Exh) when power output could no longer be maintained. FFA, free fatty acids. *Significantly different from Pl within same protocol, P < 0.05.

consumption, carbon dioxide production, VO_{2\text{max}} (data not shown), or respiratory exchange ratio (RER) (Table 4). Each subject achieved ~85% VO_{2\text{max}} (49.9 ± 0.05 ml·kg^{-1}·min^{-1}) by the 20-min time point during exercise as calculated from the initial VO_{2\text{max}} test and maintained this power output until near exhaustion.

Diet. On the basis of self-reported diet records, there were no significant differences between subjects and between trials for a given subject with respect to the percentage of dietary fat, carbohydrate, and protein. Subjects consumed a mixed diet of 30–35% fat, 50–54% carbohydrate, and 12–14% protein. Subjects reported that they maintained the same diet immediately before each trial.

Perception of trial substance. When asked by questionnaire to identify which substance they had received, subjects generally correctly identified the trial substances, with some exceptions. One subject was unable to discriminate between caffeine and placebo on most occasions (incorrect in 5 of 7 trials). Another subject did identify caffeine correctly but was unable to definitely identify placebo during periods of withdrawal. The subjects’ justification for guessing caffeine included feelings of alertness, less fatigue during exercise, caffeine-induced diuresis, and talkativeness. The withdrawal symptoms varied in severity among subjects and between similar withdrawal periods.

DISCUSSION

In this study the effects of short-term withdrawal from dietary caffeine during high-intensity exercise on hormonal, metabolic, and endurance responses with or without acute ingestion of caffeine were examined. The present study is the first to investigate the effect of...
withdrawal from dietary caffeine over the critical 4-day period in which tolerance to caffeine and withdrawal symptoms are reported (27), and it is unique in the examination of the influence that prior caffeine use may have on epinephrine and ergogenic responses during high-intensity exercise. Under the placebo condition, days of withdrawal, i.e., 0, 2, or 4 days, did not alter the basic responses in any measured parameters. Withdrawal from caffeine resulted in caffeine-induced changes in serum FFAs at 4 days and in plasma NE responses at 2 and 4 days. The significance of the changes in concentration of these metabolites after acute caffeine ingestion is uncertain because withdrawal from dietary caffeine did not alter either the ergogenic or the epinephrine response.

When the exercise literature is reviewed, there is inconsistency found regarding the ergogenic effect of caffeine that may be attributable to lack of control of prior caffeine use in the subjects. In two studies in which a positive ergogenic effect is reported (18, 29), a 48-h withdrawal from dietary caffeine was used before testing. In other studies, when prior caffeine use is not reported, some authors found no effect (6, 13, 28) and others a positive response (10, 14, 22). The similar longer exhaustion times in the caffeine trials in all subjects reported here suggests that withdrawal from caffeine before testing was not a factor in this variation. The mean time to exhaustion in the Pl R trial completed in this study was not significantly different from that in the Caf 0d as a result of one subject almost doubling his exercise time during the Pl R trial vs. the Caf 0d. This suggests that variation in this time to exhaustion in studies in which a small number of subjects were used may have influenced previous results. To minimize confounding variables, our subjects regulated their diet and exercise activity for 48 h before testing during all phases of withdrawal (0, 2, and 4 days) and then were tested by using standardized exercise trials. Exercise performance data throughout all days of withdrawal in the placebo trials were similar, indicating changes in the caffeine trials were a result of acute caffeine ingestion and were not a product of the protocol.

In many exercise studies, the pharmacological properties of caffeine regarding tolerance to the metabolic effects have not been considered. Adenosine receptors are widely distributed throughout the body in the brain, adipose tissue, smooth and cardiac muscle, and adrenal gland. If caffeine acts primarily as an adenosine-receptor antagonist in vivo, then short-term withdrawal should result in significant enhancement (upregulation) (24), suggesting that adenosine-mediated effects will be more pronounced. In contrast to findings in resting subjects, we demonstrate here an increase in plasma epinephrine in response to exercise and caffeine regardless of the state of habituation or withdrawal. Robertson et al. (27) found that prior caffeine use resulted in attenuation of the caffeine-induced increase in plasma epinephrine concentration, and a 4-day period of abstinence was used to restore the expected epinephrine increase after acute caffeine ingestion. On the basis of this literature and other research involving adenosine-receptor-mediated responses (4), we determined that a 2- and a 4-day withdrawal would be adequate to observe any metabolic and hormonal changes that occur during the transition from habituation through withdrawal. The lack of epinephrine response to withdrawal after acute caffeine ingestion reported here both at rest and during exercise may be due to the exercise intensity. It appears that the strong sympathetic stimulus of exhaustive exercise during habituation and withdrawal acts to surmount tolerance to the caffeine stimulus, which contributed to the uniform rise in plasma epinephrine at exhaustion.

Table 3. Mean plasma caffeine data

<table>
<thead>
<tr>
<th>Treatment</th>
<th>-60</th>
<th>0</th>
<th>5</th>
<th>20</th>
<th>Exh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pl 0d</td>
<td>10.99 ± 3.09</td>
<td>9.30 ± 2.72</td>
<td>9.95 ± 2.87</td>
<td>9.04 ± 2.70</td>
<td>8.47 ± 2.25</td>
</tr>
<tr>
<td>Pl 2d</td>
<td>1.09 ± 0.49</td>
<td>1.36 ± 0.63</td>
<td>1.02 ± 0.53</td>
<td>1.36 ± 0.70</td>
<td>0.89 ± 0.44</td>
</tr>
<tr>
<td>Pl 4d</td>
<td>0.78 ± 0.51</td>
<td>0.43 ± 0.30</td>
<td>0.42 ± 0.26</td>
<td>0.58 ± 0.38</td>
<td>0.41 ± 0.29</td>
</tr>
<tr>
<td>Caf 0d</td>
<td>7.11 ± 2.26</td>
<td>36.66 ± 6.28</td>
<td>50.39 ± 6.93</td>
<td>50.72 ± 6.27</td>
<td>50.69 ± 8.15</td>
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<tr>
<td>Caf 2d</td>
<td>3.15 ± 1.56</td>
<td>32.87 ± 2.76</td>
<td>41.63 ± 2.42</td>
<td>39.09 ± 3.11</td>
<td>35.28 ± 4.79</td>
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<tr>
<td>Caf 4d</td>
<td>0.43 ± 0.27</td>
<td>39.24 ± 5.15</td>
<td>40.77 ± 5.61</td>
<td>44.43 ± 5.86</td>
<td>41.18 ± 5.63</td>
</tr>
</tbody>
</table>

Values are means ± SE in µM for 6 subjects. Ingestion of caffeine resulted in significantly greater concentration vs. placebo.

Table 4. Mean RER data

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>10</th>
<th>15</th>
<th>20</th>
<th>Exh</th>
</tr>
</thead>
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<td>Pl 0d</td>
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<td>0.85 ± 0.02</td>
<td>0.84 ± 0.01</td>
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<tr>
<td>Pl 2d</td>
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<td>0.86 ± 0.01</td>
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<td>0.85 ± 0.01</td>
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<td>Pl 4d</td>
<td>0.85 ± 0.02</td>
<td>0.84 ± 0.02</td>
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<td>Caf 0d</td>
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<tr>
<td>Caf 2d</td>
<td>0.86 ± 0.01</td>
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<td>Caf 4d</td>
<td>0.87 ± 0.02</td>
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<td>0.83 ± 0.02</td>
<td>0.81 ± 0.03</td>
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Values are means ± SE for 6 subjects. RER, respiratory exchange ratio.
regardless of dietary caffeine use before experimenta-
tion. An alternative explanation for the changes in epi-
 nephrine and NE concentrations at exhaustion in the
caffeine trials would include a linear relationship be-
tween time to exhaustion and increases in plasma
catecholamines rather than a caffeine effect. In the
present study, it is impossible to determine whether the
effect on catecholamines is merely a result of a longer
duration of exercise. However, in examining the data,
epinephrine concentrations are similar between the
Caf 0d trial and the Caf 4d trial, despite the latter
lasting 7 min longer. The changes in NE are more
difficult to interpret because of the similarity of time
between 2- and 4 day trials. Because of the uncertainty
of interpreting catecholamine data from this study,
future research will be needed to determine the rela-
tionship between caffeine-induced changes in epineph-
rine concentration during exercise and time to exhaus-
tion. Regardless, the number of days of withdrawal
from caffeine did not influence the ergogenic effects of
acute caffeine ingestion, and the mechanism for this
may be related to a number of factors.

These data indicate that acute caffeine ingestion
during short-term withdrawal alters mobilization of
serum FFA. The changes in serum concentrations after
withdrawal from caffeine may not have altered uptake
or oxidation because there was no significant impact on
RER, but without arteriovenous data this remains
speculative. Our data are in agreement with those
reported by Hetzler et al. (21), who found that acute
withdrawal from caffeine did not alter substrate utiliza-
dation during submaximal exercise. However, these inves-
tigators limited their study to a 48-h interval to test
withdrawal, which we believed was not sufficient be-
cause there is considerable variability in the reported
time course of symptoms of withdrawal from 12 to 48 h
(20). In a study by Biaggioni et al. (4), a 60-h with-
drawal was required to alter adenosine-mediated plate-
let response to methylxanthines in human subjects.
This proved to be the case with respect to serum FFA
responses in our subjects, in whom there was a signifi-
cant caffeine effect after a 4-day withdrawal, which
would have been missed if only 2 days were investigat-
ed.

To counteract the possibility of a statistical error due
to multiple comparisons, the Bonferroni corrected t-test
was applied, increasing the rigor of acceptance of the
null hypothesis. Therefore, the significant changes in
both FFA at rest and NE during exercise in the present
study are likely a result of withdrawal from caffeine.
Whether the absence of enhanced substrate utilization
would have continued over 4 days in the work of
Hetzler et al. (21) is not known, and the lack of
methylxanthine data in their research makes interpre-
tation of prior caffeine status impossible to establish.
However, these data, coupled with the present findings,
suggest that the mechanism through which caffeine
acts to enhance exercise performance may not be linked
to changes in adipose lipolysis. The increase in lipolysis
may be related to the direct systemic effects of caffeine
ingestion (31) through action on adenosine receptors
rather than through secondary stimulation via the
sympathetic nervous system.

The complexity of the metabolic responses to acute
caffeine ingestion during exercise makes dissociation of
the possible causal effects difficult to distinguish. Distrib-
ution of adenosine receptors throughout the periph-
ERAL tissues and the central nervous system suggests
that caffeine may act directly or indirectly through
multiple mechanisms (4, 17, 25, 31). Additional support
for the direct action of caffeine on specific tissues can be
found in several studies (25, 32). Lopes et al. (25) used
electrical stimulation of the adductor pollicis after
ingestion of caffeine to dissociate both metabolic and
centrally mediated effects from increases in time to
fatigue. Subsequent research is needed to determine
whether it is peripheral vs. centrally mediated effects
that are significant in the caffeine-mediated ergogenic
response.

To investigate whether our results could be influ-
enced by variations in available plasma concentrations
of methylxanthines, the measurements of caffeine con-
centrations before and during exercise in this study
were used to confirm compliance to the protocol and as
an indication of the similarity of drug stimulus between
trials. Despite high plasma caffeine concentrations in
the Pl 0d trial (~10 µM), changes in performance did not
occur until greater concentrations (>30 µM) were
achieved in the caffeine trials. Graham and Spriet (19)
report that plasma caffeine concentrations of 18 µM
(after ingestion of 3 mg/kg caffeine) result in ergogenic
effects. Whether there is a critical concentration be-
 tween 10 and 18 µM that represents a threshold of
caffeine activity during exercise is not known.

Withdrawal from dietary caffeine did not alter the
maximal plasma caffeine concentration during exercise
despite the presence of higher initial concentrations in
the no-withdrawal trial. This is consistent with the
data available regarding caffeine metabolism in the
liver. The cytochrome P-450 isofrom 1A2, which repre-
sents the major pathway for caffeine 1-and 7-demethyl-
ations (23), is saturable (12), and steady-state concen-
trations were achieved over the time course in the
present study. Factors that would alter the activity of
the P450 1A2 enzyme include smoking, oral contracep-
tive use, ingestion of cruciferous vegetables, and physi-
ical fitness (5, 33). Subjects in the present experiment
did not smoke or use oral contraceptives, and diet
analysis indicated no use of cruciferous vegetables.
They maintained the same level of fitness throughout
the experiment. Therefore, these factors would have
minor, if any, effect on available plasma concentrations
of caffeine and are unlikely to have influenced findings
in this study. Subjects arrived after a 4-day withdrawal
from caffeine with small, but measurable, caffeine
concentrations, which is consistent with the wide vari-
ability in caffeine half-life (1.5–9.5 h) (21). The minor
increase of 15–20% in plasma caffeine concentration in
the Caf 0d vs. the Caf 2d or Caf 4d trials did not influ-
ence results reported here, which is in agreement
with Graham and Spriet (19), who found no difference
in performance between ingestion of 3 vs. 6 mg/kg of caffeine before exercise.

The ability to discriminate caffeine has been tested in humans and animals (20, 24). Because of the effects of caffeine in humans on arousal and awareness, we recorded our subjects’ observations of their trial substances. The majority of subjects correctly identified caffeine by the effects of arousal and alertness, and, therefore, it is difficult to claim that they did not know which trial substance had been given. The effect on their performance, therefore, may have been influenced in some way by knowledge that they had received caffeine; however, the consistently longer exercisetimes to exhaustion in the caffeine trials for all subjects, even those who guessed incorrectly, suggests that this was not a significant factor in these results.

All of the subjects tested in the present study subjectively reported withdrawal symptoms lasting from 2 to 4 days. These included severe headaches, fatigue, lethargy, and flu-like symptoms, indicating that they were experiencing a reversal of the effects of habituation to caffeine. In animals and humans, the symptoms of withdrawal are thought to be associated with upregulation of adenosine receptors after prolonged caffeine administration (4, 24). Therefore, it would be expected that metabolic and hormonal responses in humans mediated through adenosine receptors would be altered during short-term withdrawal from caffeine. The significant increase in serum FFAs at rest and the increased plasma NE responses after a 2- and 4-day withdrawal, and the trend for increases in whole-blood lactate during withdrawal are indicative of an alteration in sensitivity in the metabolic responses to acute caffeine ingestion during withdrawal. Whether the ergogenic response after caffeine ingestion is mediated by increases in plasma epinephrine has yet to be determined. However, the dissociation between these metabolic and plasma epinephrine responses suggests that caffeine acts directly on specific tissues rather than through epinephrine-mediated responses. Several researchers (7, 31) have demonstrated a dissociation between caffeine-induced epinephrine and FFA responses, and the data reported here support these findings. However, care must be taken in interpreting the results presented here because of the relative insensitivity of venous blood measurements, and turnover data would be necessary to fully understand this relationship. Despite the need for more detailed data, there are reasonable internally consistent data to suggest that a possible change in sensitivity at the level of peripheral or central adenosine receptors is the mechanism of action for the ergogenic response to acute caffeine ingestion.

In summary, variable periods of short-term withdrawal from dietary caffeine had no effect on caffeine-induced increases in endurance during high-intensity exercise compared with no withdrawal. There were variable responses to acute caffeine ingestion during withdrawal on FFAs and NE but no effect on plasma epinephrine or RER. We conclude the mechanism through which caffeine acts as an ergogenic aid is unlikely to be through changes in available metabolic substrates or catecholamines but rather is through some direct action of caffeine on tissues as yet to be described.

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